STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR CHICKEN GONADOTROPIN-RELEASING HORMONE II ANALOG (GnRH IIa) (INAD 13-345)

Sponsor:

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

_____ _____

Sponsor Signature Date Approved

Manufacturer:

DelTaq Fish Health LLC
PO Box 343
Stoneville, MS 38776

Office for Coordination of GnRH IIa INAD:

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

Proposed Starting Date:	April 1, 2020
Proposed Ending Date:	July 30, 2028
Study Director:	Bonnie Johnson

Clinical Field Trial Location:

Facility:_____

Investigator:_____

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STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR CHICKEN GONADOTROPIN-RELEASING HORMONE II ANALOG (GnRH IIa) UNDER INAD #13-345

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of gonadotropin releasing hormone II analogue (GnRH IIa) proposed for use as a spawning aid for female ictalurids under INAD 13-345.

II. SPONSOR

Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: marilyn_j_blair@fws.gov

Manufacturer: DelTaq Fish Health LLC
PO Box 343
Stoneville, MS 38776
Email: tomg@deltaqfishhealth.com

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

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program
4050 Bridger Canyon Road, Bozeman, MT 59715;
Phone: 406-994-9911; Email: paige_maskill@fws.gov

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES

Proposed Starting Date: March 13, 2020

Proposed Completion Date: July 30, 2028

V. BACKGROUND/PURPOSE

A. Background Information:

Aquaculture presents an opportunity to sustainability increase production of fish, both for conservation purposes and to satisfy the global fish consumption of a growing human population. However, one of the most serious limitations in the advancement of commercial aquaculture of teleost fish species is the control and management of reproductive processes in captivity (Zohar and Mylonas, 2001). Though many advances have been made in husbandry and captive rearing of finfish species, many still exhibit reproductive dysfunctions when captive spawning is attempted. Most commonly, females fail to undergo final oocyte maturation and thus ovulation and spawning. Therefore, the ability to manipulate and control teleost fish reproduction in captivity will not only significantly improve the industry's ability to provide a steady supply of fish, both in on and off-season spawning, but also allow selective genetic manipulations to further enhance the growth, survival, and flesh quality characteristics of teleost fish reared in captivity. The outcome is increased quantity and quality of fish, both for human consumption and conservation measures.

In general, finfish fail to reproduce in captivity due to reproductive dysfunction caused by stressors associated with the captive environment. As reviewed in Zohar and Mylonas (2001), in females, this is frequently the result of three types of dysfunction:

1. Failure to mature at all (i.e. vitellogenesis does not occur);
2. Absence of final oocyte maturation (i.e. vitellogenesis occurs, but does not progress through final oocyte maturation, ovulation, and spawning);
3. Maturation occurs but spawning does not.

While these reproductive dysfunctions may be addressed through environmental manipulations (i.e. temperature, photoperiod), such efforts alone are often not sufficient to fully overcome the physiological impacts captivity has on limiting sexual maturation and thus reproductive success. Peptides such as Gonadotropin Releasing Hormone analogs (GnRHa, which include GnRH IIa), provide a safe, effective approach to overcome these physiological dysfunction(s) and enhance sexual maturity and spawning of female finfish in captivity.

Benefits of GnRHa (reviewed in Zohar and Mylonas 2001) are that these peptides induce the release of endogenous hormones (e.g. LH/FSH) and repair the endocrine disruption that is causing the reproductive dysfunction. Further, GnRHa act at an elevated level on the Hypothalamus-Pituitary-Gonad (HPG) axis and provide stimulation directly to the pituitary to induce sexual maturation. In addition, unlike other spawning aids (e.g. Carp Pituitary), GnRHa can easily be synthesized and purified, thus presenting no risk of pathogen transmission. Finally, GnRH IIa display a

unique structure present in all teleost fish species, and thus the use of GnRH IIa can be successfully applied with success to a wide range of teleost fish species.

B. Purpose of INAD:

The purpose of this INAD for GnRH IIa in a liquid form (saline) for a two phase injection is to develop clinical field trial data that will be used to determine the efficacy and appropriate treatment regimens for use as a spawning aid for female ictalurids. These data will be used to support a new animal drug application (NADA) for "GnRH IIa".

The U. S. Fish and Wildlife Service (USFWS) and Aquatactics anticipate that it may take several years to complete all the technical section data for a NADA for GnRH IIa. The USFWS and Aquatactics believe it is likely that data from 5-7 treatment seasons will be required in order to adequately assess the efficacy of GnRH IIa as a spawning aid for female ictalurids 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 GnRH IIa as a spawning aid in cultured female ictalurids under typical hatchery situations.

2. Provide the opportunity for fish culturists and fisheries managers to legally use GnRH IIa 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 for GnRH IIa use in female ictalurids.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Common Name: GnRH IIa or cGNRH IIa

Chemical Name: (Des-Gly10, D-Arg6, Pro-NHEt9)-LHRH II

(CHICKEN)

CAS Number: 145940-57-4

Amino Acid Profile: pGlu-His-Trp-Ser-His-D-Arg-Trp-Tyr-Pro-NHEt

Appearance: White lyophilized powder

Clear liquid when resuspended in saline

b. Strength and dosage form

GnRH IIa is a synthetic peptide analogue of gonadotropin releasing hormone II (GnRH II). It is presented as a dry powder to be resuspended in physiological saline solution right before use for an intraperitoneal injection. Dilution rate is dependent upon fish size and intended dosage.

c. Manufacturer, source of supply

DelTaq Fish Health LLC
PO Box 343
Stoneville, MS 38776

Contact Person: Tom Goodrich/Rebecca Goyt
Phone: 425-629-8099
Fax: 425-629-8095:
email: tomg@deltaqfishhealth.com or rebeccag@aquatactics.com
website: www.deltaqfishhealth.com

2. Verification of Drug Integrity/Strength:

The Manufacturer will provide the analytical data necessary to establish the purity of each lot of GnRH IIa supplied. The lot number and date of manufacture for each batch of GnRH IIa 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 GnRH IIa-1) will clearly identify the lot number for all GnRH IIa shipments. If the integrity of the GnRH IIa 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 GnRH IIa-2). All un-usable GnRH IIa must be destroyed by following the disposal methods described in the SDS. The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

GnRH IIa will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. The container will be stored frozen (-20^{O}C) and out of direct sunlight. The storage unit (i.e. most likely a manual defrost freezer) must be labeled to indicate that it contains hazardous material and that "*NO Food or Drink is to be Stored in this Freezer*". GnRH IIa 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 GnRH IIa (see Appendix IV). Each person involved with the study and each person who may be present during the use of GnRH IIa shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with GnRH IIa.

5. Investigational Labeling

A copy of the label to be attached to each container of GnRH IIa are provided in Appendix V. Although investigational labels will be affixed to GnRH IIa containers by the supplier, it is the responsibility of the Investigator to ensure proper labeling of all containers of GnRH IIa.

6. Accountability

AquaTactics will be the sole supplier of GnRH IIa to all Investigators under this INAD.

The Online INAD Database must be used by Investigators for ALL INAD reporting. The online INAD database 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 online INAD database (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the online INAD database) investigators must archive hard copies of all raw data.

1. All facilities Using GnRH IIa:

Immediately upon receiving an order/shipment of GnRH IIa, the Investigator will complete Form GnRH IIa-1 “Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (located in the “Manage/View Drug Inventory” section of the investigator account). The Study Director will forward a copy of this form to the FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form GnRH IIa-1s are received by the Study Director within 10 days of drug receipt.

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

At the conclusion of field trials, all remaining GnRH IIa will be disposed of by following the disposal methods in the Safety Data Sheet (note: unless medicated feed is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all GnRH IIa must be properly recorded and accounted for on

the Chemical Use Log (Form GnRH IIa-2). The Study Monitor will be responsible for verifying the quantity of GnRH IIa remaining on hand versus the amount indicated on Form GnRH IIa-2. **Note:** GnRH IIa can be transferred to other facilities that are participating under INAD 13-345. Transfers must be shown in the Drug Inventory section of the database (formerly Form GnRH IIa-2).

7. Preparation Procedures

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

10mL of sterile physiological saline solution can be added to the 25mg vial to obtain a Stock Solution concentration of 2500 µg/mL.

To obtain 50mL at 20 µg/mL, dilute 0.4mL of Stock Solution into 49.6mL of sterile physiological saline solution.

To obtain 50mL at 80 µg/mL, dilute 1.6mL of Stock Solution into 48.4mL of sterile physiological saline solution.

Inject 1mL per kg of body weight

Recommended dose of 100 µg/kg as two injections (20% then 80%) is sufficient to act as a spawning aid in female ictalurids. Administer to fish as an intraperitoneal (IP) injection using a sterile syringe and needle (18-20 g needle recommended).

B. Items Needed for Treatment, Data Collection, Etc.:

Treatment equipment should include a scale to determine fish weight and appropriate size sterile needle and syringe (18-20 g needle recommended).

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the GnRH IIa INAD will need to complete several forms. These forms are described in Section XIII. Copies of these forms are attached to this Study Protocol and will be used as a guide only for collecting the data that will be entered into the online INAD database.

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, it is strongly encouraged that whenever possible, the experimental unit in clinical field trials is individual animals**. Whenever 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 for the current calendar year before GnRH IIa can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor or by an Investigator to address emergency-use situations (See Section XX). However, poor planning and/or a lack of preparation will not be considered an emergency situation.

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

C. Period of use

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

D. Environmental conditions

Since GnRH IIa will be injected directly into the peritoneal cavity, there will be no drug discharge from participating facilities. Therefore, GnRH IIa qualifies for a categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.33(e).

Environmental conditions will be variable and include a spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on Form GnRH IIa-3. Drug discharge must be in compliance with local **NPDES** permitting requirements.

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 GnRH IIa-W: “Worksheet for Designing Individual Field Trials” (located under the “New Study Request” tab in the investigator account) that pertains to each specific treatment event. The worksheet should be filled out and forwarded to the Study Monitor through the online INAD database. The Study Monitor will review the planned treatment (worksheet) 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 the online INAD database will 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 any paper forms that are being used as a guide to collect the data to enter in the online database (i.e., Form GnRH IIa -2 and GnRH IIa -3), as well as on any additional correspondence regarding that specific treatment event. If for some

reason the Investigator is unable to reach the Study Monitor with regards to Worksheet approval and the need for treatment is immediate, the Investigator should contact the AADAP Office for permission to proceed.

Note: The online INAD database, which 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. However, **the experimental unit should be considered individual fish whenever possible**.

B. Control groups will not be a requirement for clinical field trials evaluating the efficacy of GnRH IIa treatment. In some cases the number of broodfish available at a given time for GnRH IIa 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 would be difficult.

C. Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, **control groups are strongly encouraged whenever circumstances permit**. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse effects 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.

D. Although as stated above untreated control groups are not a required element of treatment under this INAD exemption, **it is important for all investigators to note that field trials conducted under a more stringent study protocol (i.e including requirements for non-treated controls groups, replication, blinding, dose verification, etc) will ultimately be required in order to support a NADA for GnRH IIa. It is also important to note that the INAD sponsor fully expects that a limited number of facilities/investigators listed under this INAD exemption will agree to participate in such "pivotal" efficacy studies.** These studies will be initiated only after direct consultation between facilities/investigators and the sponsor. These studies will be conducted under a separate FDA-approved study protocol (i.e. not the INAD study protocol), and will also be conducted with assistance from, and under the direct supervision of, the sponsor. **If for any reason it becomes apparent to the sponsor that facilities/investigators listed under this INAD are not willing to participate in such "pivotal" studies, the sponsor will request that FDA terminate the INAD.**

XI. TREATMENT SCHEDULES

A. Route of administration

GnRH IIa should be brought to room temperature and injected into the coelomic cavity using an appropriately sized sterile needle and syringe (18-20g). Injections should be administered in the peritoneal cavity. Dilutions of hormones are calculated so each fish receives 1mL per Kg. Insert the GnRH IIa needle, while holding the fish firmly, inject cavity laterally to the uro-genital area under the pelvic fins. Discharge the contents of the syringe and remove the needle. It is strongly encouraged that all fish be anesthetized prior to injection.

B. Dose to be administered

Standard hormone dosage rates will be no more than 100 ug/kg body weight.

C. Dosing interval and repetition

GnRH IIa will be administered in a 2 steps manner, 20% of the dose during the first injection and 80% of the dose during the second injection at 12-24h interval.

D. Drug preparation procedures

GnRH IIa will be supplied by DelTaq Fish Health LLC as a sterile, lyophilized powder in a multi-use container. GnRH IIa will be supplied in 25mg/vial.

10mL of sterile physiological saline solution can be added to the 25mg vial to obtain a Stock Solution concentration of 2500 µg/mL.

To obtain 50mL at 20 µg/mL, dilute 0.4mL of Stock Solution into 49.6mL of sterile physiological saline solution.

To obtain 50mL at 80 µg/mL, dilute 1.6mL of Stock Solution into 48.4mL of sterile physiological saline solution.

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 GnRH IIa 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 GnRH IIa treatment involved should be appropriately labeled.

An exception to this concomitant therapy is that the MS222 and Aqui-S 20E anesthetics may be used to sedate fish prior to GnRH IIa treatment. If an anesthetic is used please note which one was used in Form GnRH IIa-3 under the description of results section. Note: the withdrawal time must be followed for whichever anesthetic was used.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begin 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 GnRH IIa-3. Treatment response parameters that should be addressed include the following:

1) Primary Parameters

The primary response parameter for evaluating the effect of GnRH IIa on fish will be whether a fish is “ripe” or “non-ripe” following treatment. In the case of females, ripe fish are those that have released their eggs in response to normal artificial spawning procedures. Non-ripe fish have not released eggs. With respect to data reporting under this INAD, eggs will only be collected one time from individual fish.

2) Secondary Parameters

Secondary response parameters for females will include percent hatch.

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

3) Adverse Reactions

Any adverse reaction that occurs during the study period (whether considered/suspected to be treatment-related or not) 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 GnRHIIa has been used with beneficial effect in fish culture and a preliminary TAS has been conducted showing no effects of 5x the dose, 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.

4) Mortalities and Moribund Fish

Any fish that die or are euthanized during the study period should undergo a complete necropsy. Necropsy should include examination of the implant site. Necropsy results should be recorded on Form GnRH IIa-4N: Necropsy Report Form. If it appears that fish died due to handling stress that needs to be reported in the GnRH IIa Form 3: Results Report.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the GnRH IIa INAD will need to complete the following forms:

Form GnRH IIa-W. Worksheet for Designing Clinical Field Trials under INAD 13-345 - located in the New Study Request tab

Form GnRH IIa-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals - located in the Manage/View Drug Inventory tab

Form GnRH IIa-2. Drug Inventory Form for use of GnRH IIa under INAD 13-345 - located in the Manage/View Drug Inventory tab and filled out in Form GnRH IIa - 3 to show use

Form GnRH IIa-3. Results Report Form for use of GnRH IIa under INAD 13-345 – located in the Active Studies table on the home page

Form GnRH IIa-4N. Necropsy Report - located in Form GnRH IIa - 3

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

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located in the online INAD database. All current and completed studies conducted under the investigator account will be stored and available in the online INAD database to the current study monitor, study investigator, and AADAP.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. Fish will be able to be released 1 day after last injection; follow the MS222 or Aqui-S 20E withdrawal time if it is used to sedate fish at time of injection.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

GnRH IIa will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or outdated GnRH IIa remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Drug disposal information is available in the Safety Data Sheet (SDS) located in Appendix IV of this protocol. Disposition of all GnRH IIa must be properly recorded and accounted for on the Chemical Use Log (Form GnRH IIa -2). The Study Monitor will be responsible for verifying the quantity of GnRH IIa remaining on hand versus the amount indicated on Form GnRH IIa-2. The investigational drug may not be redistributed to others not specified by the protocol and should not be retained by the Investigator after completion of the study (note: unless GnRH IIa is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer).

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

A. Drug distribution

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

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor will be selected by each facility that is authorized to treat fish with GnRH IIa. 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 GnRH IIa 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.).

D. Administrator of the drug

GnRH IIa will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). GnRH IIa 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 protocol Section VII.A.6. Accountability for details and the following forms will be used as guides for data collection: Forms GnRH IIa-W, GnRH IIa-1, GnRH IIa-2, GnRH IIa-3, and GnRH IIa- 4N.

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. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

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. **Note:** data that is entered through the online INAD database will be archived in the database. These archived forms will be available as long as the study participant accounts remain open.

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. INAD reports will be prepared and submitted to the FDA as required. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

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

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

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation**. Deviation statements should be documented on Form GnRH IIa-3 in the *Description of Results* section and in the *Study Deviation* field.

XXI: E.O. 13891

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency policies.

LITERATURE CITED

Zohar, Yonathan & Mylonas, Constantinos. (2001). Endocrine manipulations of spawning in cultured fish: From hormones to genes. Aquaculture. 197. 99-136. 10.1016/S0044-8486(01)00584-1.

Appendix I. Sponsor Contact Information for GnRH IIa INAD #13-345

Sponsor: Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9904
Fax: (406) 582-0242
Email: marilyn_j_blair@fws.gov

Sponsor Address: 4050 Bridger Canyon Road, Bozeman, MT 59715

Study Director: Ms. Bonnie Johnson
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9905
Fax: (406) 582-0242
Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9911
Fax: (406) 582-0242
Email: paige_maskill@fws.gov

Appendix II. Study Monitors for GnRH IIa INAD #13-345

Note: This information will be provided directly to CVM

Appendix IIIa. Facilities and Names of Investigators Participating under GnRH IIa INAD #13-345

Note: This information will be provided directly to CVM and DelTaq Fish Health LLC

Appendix IIIb. Sample of Knowledge Required for Position of Hatchery Manager (i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal, State, Tribal, and private agencies/facilities.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.

Appendix IV. Safety Data Sheet (SDS) for GnRH IIa INAD #13-345

See the following 8 pages for the SDS from the supplier.

SAFETY DATA SHEET

SECTION 1: Identification of the substance

1.1 Product identifiers

Product name : GnRHIIa (Gonadotropin Releasing Hormone II Analogue)

(Des-Gly^{10}, D-Arg^{6}, Pro-NHEt^{9})-GnRHII

Product Number : 013060
Brand : DelTaq Fish Health
CAS-No. : 145940-57-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : For Use as Spawning Aid in Female Ictalurid Catfish

1.3 Details of the supplier of the safety data sheet

Company : DelTaq Fish Health LLC

Telephone : +1 425 629 8099
Fax : +1 425 629 8095

1.4 Emergency telephone number

Emergency Phone # : CHEMTREC 24 HOUR EMERGENCY NUMBER:
1-800-424-9300

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Reproductive toxicity (Category 2), H361

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram 

Signal word Warning

Hazard statement(s) Suspected of damaging fertility or the unborn child.
H361

Precautionary statement(s)

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P281	Use personal protective equipment as required.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula : $C_{64}H_{79}N_{19}O_{12} . xC_2H_4O_2 . yH_2O$
Molecular weight : 1,306.44 g/mol
CAS-No. : 145940-57-4

Component	Classification	Concentration
(Des-Gly[10], D-Arg[6], Pro-NHEt[9])-GnRHII	Repr. 2; H361	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures General Advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Carbon oxides, Nitrogen oxides (NOx)

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.
Evacuate personnel to safe areas. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.
Recommended storage temperature -20 °C
Storage class (TRGS 510): 6.1D: Non-combustible, acute toxic Cat.3 / toxic hazardous materials or hazardous materials causing chronic effects

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters

Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment Eye/face protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. 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.

Body Protection
Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full- face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Appearance	Form: solid
b) Odor	No data available
c) Odor Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	No data available
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapor pressure	No data available
l) Vapor density	No data available
m) Relative density	No data available
n) Water solubility	No data available
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)
Other decomposition products - No data available. In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity: No data available

Inhalation: No data available

Dermal: No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
Presumed human reproductive toxicant May cause reproductive disorders.
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport

information DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

SECTION 15: Regulatory

information SARA 302

Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Chronic Health Hazard

SECTION 16: Other

information Further

information
The above information is believed to be correct but does not claim to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. DelTaq Fish Health shall not be held liable for any damage resulting from handling or from contact with the above product.

Appendix V. Investigational Label for GnRH IIa INAD #13-345

1. Investigational label for tests in vitro and in laboratory research animals [511.1(a)]:

   "Caution. Contains a new animal drug for investigational use only in laboratory animals or for tests in vitro. Not for use in humans."

2. Investigational label for use in clinical field trials [511.1(b)]:

   "Caution. Contains a new animal drug for use only in investigational animals in clinical field trials. Not for use in humans. Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture."

Appendix VIa. Fish Species Treated under GnRH IIa INAD #13-345

Female Ictalurids (catfish)

Appendix VIb. Table of Facilities and Fish Stocks Treated under GnRH IIa INAD #13-345

Note: This information will be provided directly to CVM

All data must be entered through the online INAD database:

The following forms are to be used as a guide for collecting data that will be entered into the **online INAD database**. Any paper forms that are submitted to AADAP will be sent back to the study participants.

Form GnRH IIa-W: Worksheet for Designing Clinical Field Trials under GnRH IIa INAD 13-345

INSTRUCTIONS

1. Investigator must fill out Form GnRH IIa-W for each trial conducted under this INAD **before** actual use of GnRH IIa.
2. Investigator should forward a copy of GnRH IIa-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

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

		Fish species to be treated		
Average fish size (in)			Average fish weight (gm)	
Number of treated females			Number of control females	
Anticipated date of treatment			Estimated total amount of drug for proposed treatments (mg)	
Intended GnRH IIa dosage (µg/kg)	Females		Method of administration	IP Injection
Number of injections per fish			Injection Interval (hrs)	
Drug manufacturer	DelTaq Fish Health LLC		Drug lot number	

STUDY DESIGN: Provide a brief description of your planned study. The description should include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by; _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

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

☐ Investigator or alternate shall 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 the Safety Data Sheet for GnRH II analog and have been provided protective equipment, in good working condition, as described in the SDS.

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

Form GnRH IIa-1: Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

1. Investigator must fill out Form GnRH IIa-1 **immediately** upon receipt of GnRH IIa.
2. Investigator should forward a copy of Form GnRH IIa-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 in triplicate:

Name of Drug	**GnRH IIa**	INAD Number	**13-345**
Proposed Use of Drug	To act as a spawning aid in a variety of fish species.		
Date of CVM Authorization Letter	03/12/2020		
Source of Drug	DelTaq Fish Health LLC		
Date of Drug Receipt		**Amount of Drug Received**	
Drug Lot Number		**Study Worksheet Number**	
Name of Investigator			
Address of Investigator			
Location of Trial			
Approximate Number of Treated Animals			
Study Protocol Number	13-345		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum total dose	100 µg/Kg body weight		
Methods(s) of Administration	IP Injection		
Withdrawal Period	1 day after last injection; follow MS222 or Aqui-S 20E withdrawal time if it is used to sedate fish at time of injection		

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

Date Reviewed: _____ **Sponsor:** _____

Form GnRH IIa-2: Drug Inventory Form

For Use in GnRH IIa Clinical Field Trials Conducted under GnRH IIa INAD 13-345

INSTRUCTIONS

1. Investigator should initiate a new form GnRH IIa-2 **immediately** upon receipt of each shipment of GnRH II analog.
2. Each lot number of GnRH IIa may be used for multiple treatment regimens.

Qty of GnRH IIa from previous page (mg) _____ Facility _____ Reporting individual _____

Date	Amount of new GnRH IIa received (mg)	Lot number of GnRH IIa received	Study Number	Amount of GnRH IIa used in treatment (mg)	GnRH IIa transferred (mg)	GnRH IIa discarded (mg)	GnRH IIa remaining on hand (mg)	Inventory by (initials)
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

Form GnRH IIa-3: Results Report Form
For Use in GnRH IIa Clinical Field Trials Conducted under GnRH IIa INAD 13-345

INSTRUCTIONS

1. Investigator must fill out Form GnRH IIa-3 no later than **10 days** after completion of the study period. Attach lab reports and other information.
2. If GnRH IIa 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 GnRH IIa-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

Facility	
Reporting Individual	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Drug Lot Number		Total amount drug used (mg)	
Fish species treated		Water temperature (°F)	
Drug dosage - females (µg/kg body wt)		Treatment date(s)	
Average fish weight (gm)		Average fish length (in)	
Number of treated females		Number of control females	
		Method of administration	IP Injection
Number of injections per fish		Injection Interval (hrs)	
Spawning/evaluation interval (time from treatment until spawning)		Spawning/evaluation date(s)	

Hormone Results Record

INSTRUCTIONS

1. "Ripe" females are those fish that have released their eggs in response to normal artificial spawning procedures. "Non-ripe" fish have not released eggs.
2. Use Additional copies of this form for additional treatment days

Be sure the facility name is written here: _____

GnRH IIa TREATED FISH - Females						**CONTROL FISH - Females**				
Date Treated	Date Evaluated	Ripe	Non-ripe	% Hatch		Date Evaluated	Ripe	Non-ripe	% Hatch	

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:

_____ Number of days before human consumption. Ensure this time period meets the withdrawal period described in Section XV of the Study Protocol.

_____ **NEGATIVE REPORT** GnRH IIa analog was not used at this facility under this Study Number during the reporting period. (The study will be closed out in the online INAD database.)

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

STUDY NUMBER _____

Form GnRH IIa-4N: Necropsy Report Form
For Use in GnRH IIa Clinical Field Trials Conducted under INAD 13-345

INSTRUCTIONS

1. Investigator must fill out Form GnRH IIa-4N for all fish that die or are euthanized during the study period. Use a new copy of Form GnRH IIa-4N for each individual fish.
2. Submit all Form GnRH IIa-4Ns with appropriate Form GnRH IIa-3s.

Date _____ **Fish Species/ID** _____ Fish Length (cm) _____

Evaluator(s): _____

Body surface: __ normal __ excess mucus __ irregular color __ other _____

Dermal lesion: __ none __ hemorrhagic __ other _____

__ closed __ open

Location: __ dorsal __ caudal __ ventral __ lateral __ cranial

__ base of fin - Pectoral (right), Pectoral (left), Adipose, Dorsal, Anal, or Caudal

Gills: __ normal __ pale __ hemorrhagic __ other _____

Liver: __ normal __ pale __ mottled __ other _____

Spleen: __ normal __ pale __ enlarged __ other _____

Kidney: __ normal __ pale __ swollen __ other _____

Notes and comments of gross pathologies on other organs and tissues.

eyes _____ stomach _____

body cavity _____ gastrointestinal tract _____

gall bladder _____ gas bladder _____

adipose tissue _____ musculature _____

implant site_____

other_____

_____

Investigator: _____ Date: _____