

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION
FOR SALMON GONADOTROPIN-RELEASING HORMONE
ANALOGUE (sGnRHa - OvaRH[®])
(INAD 012-186)**

Sponsor:

U.S. Fish and Wildlife Service, Division of Fish Hatcheries

Sponsor Signature

Date Approved

Manufacturer:

Western Chemical Inc.
1269 Lattimore Road
Ferndale, WA

Facility for Coordination of sGnRHa (OvaRH[®]) INAD:

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

Proposed Starting Date September 1, 2012

Proposed Ending Date August 31, 2017

Study Director Mr. Jim Bowker

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 SALMON GONADOTROPIN-RELEASING HORMONE ANALOGUE (sGnRH_a - OvaRH[®]) UNDER INAD 012-186

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of sGnRH_a (OvaRH[®]) injection to induce gamete maturation (ovulation and spermiation) in a variety of fish species. INAD 012-186.

II. SPONSOR

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

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Study Director: Mr. Jim Bowker, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9910; Fax: 406-582-0242; Email: jim_bowker@fws.gov

Principal Clinical Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: September 1, 2012

Proposed Completion Date: August 31, 2017

V. BACKGROUND/PURPOSE

The use of hormone therapy to induce spawning in fish is critical to the success of many U.S. Fish and Wildlife Service (USFWS) fisheries programs. A variety of USFWS programs, including a number that involve the restoration of threatened/endangered species, are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning. Similar use of "spawning hormones" is also critical to a wide variety of other federal, state, tribal, and private aquaculture/fisheries programs.

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). Increased cortisol levels and other inherent endocrine changes associated with spawning have a suppressive effect on the immune system that often results in increased susceptibility to a host of diseases (Maule and Schreck, 1990; Schreck, 2000). The handling required during the spawning of fish for artificial propagation complicates an already delicate situation. This is particularly true for wildstock species that must endure the added stresses of capture, handling, and confinement in an un-natural environment. The longer it is necessary to hold wild fish in captivity, the greater the likelihood of adversely affecting both the health of the fish and ultimate spawning success. In fact, with respect to many wildstock species, the stresses of capture and holding is 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 and meet recovery/restoration objectives.

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 overall fish health and potential fecundity. Successful hormone treatment can reduce handling requirements to a single hormone administration event followed by predictable 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). Investigations have found that synthetic analogues of gonadotropin releasing hormones (GnRHa) to be one of the most effective means of inducing final gamete maturation. These compounds, which may be similar to native gonadotropins found in either fish or mammals, are attractive choices as they typically exhibit both high biological activity and low species specificity. Although a number of these analogues are available, the most commonly used analogues for fish culture to date has been luteinizing hormone releasing hormone (LHRHa) and salmon gonadotropin releasing hormone (sGnRHa; Alvarino et al. 1992; Donaldson et al. 1981; Erdahl and McClain 1987; Fitzpatrick et al. 1984; Powell et al. 1995; Powell et al. 1998; Taranger et al. 1992; and Van der Kraak et al. 1983). Effective treatment has been reported using both injection and pellet implant therapy.

The use of GnRH analogues has been evaluated over the last 15 years (Crim et al., 1983a). In early attempts to use implants, peptide was imbedded in cholesterol pellets that contained cellulose to affect release rate (Sherwood et al., 1988). In this system, a 5% carboxymethyl cellulose / 95% cholesterol pellet containing mammalian GnRHa (mGnRHa) released an initial burst of mGnRHa followed by a sustained release of peptide over the next 28 days. Several

researchers have demonstrated that these types of implants were capable of inducing maturation in a variety of species including: Atlantic salmon (Crim et al., 1983a; Crim and Glebe, 1984), herring (Carolsfeld et al., 1988), sea bass (Almendras et al., 1988), rainbow trout (Crim et al., 1983b; Crim et al., 1988) and milkfish (Lee et al., 1986; Marte et al., 1988). In all of these studies, mGnRHa was the imbedded peptide that induced maturation either in advance of, or synchronously within, a population.

The inclusion of salmon GnRHa (sGnRHa) instead of mGnRHa in either implant or injection treatment designed for inducing maturation in cultured fish is a logical one. In both in vitro (pituitary fragments or cell cultures) and in vivo studies sGnRHa has been found to be more potent in effect than mGnRHa for many species including: goldfish (Peter et al., 1985, 1987), Atlantic salmon (Crim et al., 1988), rainbow trout (Crim et al., 1988; Weil et al., 1992), winter flounder (Crim et al., 1988) and catfish (Namvongchong et al., 1992b; Schulz et al., 1994). This potency may be attributed to high pituitary binding affinity and gonadotropin hormone (GtH) releasing capacity, even though sGnRH itself may not be an indigenous form for some of the species tested (Schulz et al., 1993). Moreover, sGnRHa produces a sustained level of GtH from pituitary cells with a low therapeutic dose (Peter et al., 1987). Additionally, sGnRHa either as peptide alone or as Ovaprim[®] (sGnRH + a domperidone, Syndel International, Inc.) has proven to be effective in inducing final gamete maturation in a variety of cultured fish including, but not limited to, chinook salmon (Powell, 1995), coho salmon (Powell et al., 1998), catfish (Namvongchong et al., 1992b; Schulz et al., 1993), and ricefield eel (Tao and Lin, 1993). Furthermore, sGnRHa is an attractive therapy for aquaculture use as it has been shown to be ineffective in mammals (Millar et al., 1993), and has a short half life in fish (Goren et al., 1990; Zohar et al., 1990; Weil et al., 1992). Conversely, mGnRHa is superactive in humans and has a prolonged half-life in fish and water (Sherwood and Harvey, 1986) which potentially could constitute a human safety risk. Collectively, the above-described considerations indicate that sGnRHa (OvaRH[®]) is an attractive choice for further evaluation and development as a candidate compound for a new animal drug approval for use to induce final gamete maturation in a variety of fish species.

The purpose of this compassionate INAD for sGnRHa (OvaRH[®]) injection treatment 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 sGnRHa (OvaRH[®]).

The USFWS anticipates that it may take several year to complete all technical section data for a NADA for sGnRHa (OvaRH[®]). The USFWS is aware that opportunities for sGnRHa (OvaRH[®]) therapy are unpredictable. There is no way of knowing in advance if, when, or where opportunities for pivotal studies will be encountered. The USFWS believes it is likely that data from 3-5 treatment seasons will be required in order to adequately assess the efficacy of sGnRHa (OvaRH[®]) treatment on induced gamete maturation in a variety of fish species 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 sGnRHa (OvaRH[®]) injection treatment on gamete maturation in both cultured fish under typical

hatchery situations and on critical wildstock species

2. Provide the opportunity for fishery biologists to legally use sGnRHa (OvaRH[®]) to maintain the genetic integrity and improve the reproductive potential of broodstocks during the period of time necessary for collection of efficacy, safety, and residue data required for an NADA for sGnRHa (OvaRH[®]) use in fish. Specifically, sGnRHa (OvaRH[®]) 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:	salmon Gonadotropin Releasing Hormone analogue (sGnRHa)
Product Name:	OvaRH [®]
Chemical Name:	[Des-Gly ¹⁰ , D-Arg ⁶ , Trp ⁷ , Leu ⁸] - LH-RH, ethyl amide
CAS Number:	None
Amino Acid Profile:	pGlu-His-Trp-Ser-Tyr-D-Arg-Trp-Leu-Pro-NHC ₂ H ₅
Appearance:	White fluffy crystals
Odor:	Slight musty smell

b. Strength and dosage form

sGnRHa (OvaRH[®]) is a synthetic peptide analogue of salmon gonadotropin-releasing hormone. It is presented in 10 mL sterile vials as white fluffy crystals that should be diluted with a physiological saline solution immediately prior to use. Vials contain either 1, 5, or 25 ug sGnRHa.

c. Manufacturer, source of supply

Western Chemical, Inc.
1269 Lattimore Road
Ferndale, WA 98248

Contact Person: Jim Brackett
Phone: 1-360-384-5898
Email: brackett@wchemical.com

2. Verification of drug integrity/strength:

The Manufacturer will provide the analytical data necessary to establish the purity of each lot of sGnRHa (OvaRH[®]) supplied. The lot number and date of manufacture for each batch of sGnRHa (OvaRH[®]) 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 sGnRHa/OvaRH-1) will clearly identify the lot number all of sGnRHa shipments. If the integrity of the sGnRHa (OvaRH[®]) 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 sGnRH/OvaRH-2). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

sGnRHa (OvaRH[®]) will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. The container will be stored at refrigerated temperature (~4°C) and out of direct sunlight. Stored in this manner, the shelf life of sGnRHa (OvaRH[®]) exceeds 18 months. The storage unit (i.e. most likely a refrigerator) must be labeled to indicate that it contains hazardous material and that "*NO Food or Drink is to be Stored in this Refrigerator/Freezer*". sGnRHa (OvaRH[®]) 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 sGnRHa (OvaRH[®]; see Appendix IV). Each person involved with the study and each person who may be present during the use of sGnRHa (OvaRH[®]) shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with sGnRHa (OvaRH[®]).

5. Investigational labeling

Copies of the labels to be attached to each container of sGnRHa (OvaRH[®]) are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of sGnRHa (OvaRH[®]).

6. Accountability

Western Chemical, Inc. will be the sole supplier of sGnRHa (OvaRH[®]) to all Investigators under INAD 012-186.

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of sGnRH_a (OvaRH[®]), the Investigator will complete Form sGnRH_a/OvaRH-1 "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The investigator will archive the original in the facilities INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form sGnRH_a/OvaRH-1. The Study Monitor will then forward a copy to the Study Director at the AADAP Office. The Study Director will archive one copy, and send two copies of Form sGnRH_a/OvaRH-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form sGnRH_a/OvaRH-1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of sGnRH_a (OvaRH[®]) on-hand. A Chemical Use Log (Form sGnRH_a/OvaRH-2: Drug Inventory Form) will be supplied to each Investigator. Each time sGnRH_a (OvaRH[®]) is used, it must be recorded by the Investigator on Form sGnRH_a/OvaRH-2.

7. Preparation Procedures

sGnRH_a (OvaRH[®]) for injection treatment will be supplied in 10 mL vials containing 1, 5, or 25 mg of sGnRH per vial. Immediately prior to use, sGnRH_a (OvaRH[®]) should be diluted with sterile physiological saline solution. Dilution volume will be dependent upon desired dosage, size and number of fish to be injected, and desired injection volume.

Note: based on the relatively small amount of sGnRH_a per vial (i.e., 1-25 mg), it is not recommended that Investigators attempt to create additional aliquots of sGnRH_a prior to dilution with saline.

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

Treatment equipment should include clean glassware, sterile physiological saline, a scale to determine fish weight, and 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 sGnRH_a (OvaRH[®]) 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, 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 before sGnRH_a (OvaRH[®]) 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

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

D. Environmental conditions

Since sGnRH_a (OvaRH[®]) will be injected directly into the musculature or body cavity, there will be no drug discharge from participating facilities. Therefore, sGnRH_a (OvaRH[®]) qualifies for a categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.33(e).

E. Ability of investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

Prior to initiating each treatment event, the Investigator must first complete Form sGnRH_a/OvaRH-W. "Worksheet for Designing Clinical Field Trials" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward the worksheet to the AADAP Office. The AADAP Office will review the worksheet, assign the approved treatment a Study Number, and 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. The Investigator should record the assigned study number on Form sGnRH_a/OvaRH-2, Form sGnRH_a/OvaRH-3, and Form sGnRH_a/OvaRH-4N, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, and infection/disease/treatment need is rapidly escalating, the Investigator should contact the AADAP Office for a study number and permission to proceed.

X. TREATMENT GROUPS

- A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish. 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 sGnRH_a (OvaRH[®]) treatment. In some cases, particularly with respect to wildstock populations, the number of broodfish available at a given time for sGnRH_a (OvaRH[®]) 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. It is also anticipated that species listed under the authority of the Endangered Species Act (ESA) will be treated under this INAD. With respect to species listed under the ESA, every fish may be critical to the restoration/recovery efforts.

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.

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

sGnRH_a (OvaRH[®]) should be dissolved in sterile physiological saline and administered as either an intramuscular (IM) or intraperitoneal (IP) injection.
- B. Dose to be administered

Treatment dosage will be 1-50 ug sGnRH_a per kg fish body weight.
- C. Dosing interval and repetition

Dependent upon the species/strain being treated, sGnRHa (OvaRH®) may be administered as single treatment or as a multiple treatment. Determination of whether a single or multiple treatment regimen is used will be largely a matter of past experience of the investigator and/or literature citations reporting a successful protocol(s) with respect to a specific species/strain. A multiple treatment regimen will typically consist of a single “priming” dose followed by a single “resolving” dose.

D. Drug preparation procedures

sGnRHa (OvaRH®) will be supplied in 10 mL vials containing 1, 5, or 25 mg of sGnRH per vial. Immediately prior to use, sGnRHa (OvaRH®) should be diluted with sterile physiological saline solution. Dilution volume will be dependent upon desired dosage, size and number of fish to be injected, and desired injection volume. Note: based on the relatively small amount of sGnRHa per vial (i.e., 1-25 mg), it is not recommended that Investigators attempt to create additional aliquots of sGnRHa prior to dilution with saline.

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 sGnRHa (OvaRH®) 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 sGnRHa (OvaRH®) 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 sGnRHa/OvaRH-3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

The primary response parameter for evaluating the effect of sGnRHa (OvaRH®) 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 ovulated. In the case of males, ripe fish are those undergoing active spermiation. Non-ripe fish are the obvious converse. With respect to data reporting under this INAD, eggs and milt will only be collected one time from individual fish

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:

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

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

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 sGnRH α (OvaRH $^{\text{®}}$) 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.

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 injection site. Necropsy results should be recorded on Form sGnRH α /OvaRH-4N: Necropsy Report Form.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the sGnRHa (OvaRH[®]) INAD will need to complete the following forms:

- | | |
|-----------------------|---|
| Form sGnRHa/OvaRH-W. | Worksheet for Designing Clinical Field Trials under INAD 012-186 |
| Form sGnRHa/OvaRH-1. | Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals |
| Form sGnRHa/OvaRH-2. | Drug Inventory Form for use of sGnRHa (OvaRH [®]) under INAD 012-186 |
| Form sGnRHa/OvaRH-3. | Results Report Form for use of sGnRHa (OvaRH [®]) under INAD 012-186 |
| Form sGnRHa/OvaRH-4N. | Necropsy Report Form |

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. All fish treated with sGnRHa (OvaRH[®]) must be maintained in culture facilities for at least 14 days following treatment before they may be released or allowed to enter the food chain. If fish are treated (injected) more than once, this requirement will be based on the date/time of final treatment.

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

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

sGnRH_a (OvaRH[®]) will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or out-dated sGnRH_a (OvaRH[®]) remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. The investigational drug may not be redistributed to others not specified in the Study Protocol.

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

A. Drug distribution

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

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor should be assigned to each facility that is authorized to treat fish with sGnRH_a (OvaRH[®]). 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 sGnRH_a (OvaRH[®]) itself) are already available at each participating fish hatchery. In recent years, induced final gamete maturation has become a fairly common occurrence at many broodstock facilities. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 6).

D. Administrator of the drug

sGnRH_a (OvaRH[®]) will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). sGnRH_a (OvaRH[®]) 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 sGnRH_a/OvaRH-W, sGnRH_a/OvaRH-1, sGnRH_a/OvaRH-2, sGnRH_a/OvaRH-3, and sGnRH_a/OvaRH-4N (page 11) for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on

the forms, the Investigator will send the data to the Study Monitors who will review the information and ensure that all required data is provided. The Study Monitors will in turn send the data to the 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. A copy of Form sGnRH_a/OvaRH-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Original raw data on Form sGnRH/OvaRH-2 should be retained by the Investigator until completion of the calendar year, at which time copies should be sent to the Study Monitor. Original raw data on Form sGnRH/OvaRH-3 should be retained by the Investigator until completion of the study, at which time copies should be sent to the Study Monitor. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, Study Monitors should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

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. An annual report will be prepared and submitted to the FDA. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarded to the FDA for review. Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or in a manner different than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, 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 Form sGnRH_a/OvaRH-3, and ultimately be submitted to the Study Director.

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