

**STUDY PROTOCOL FOR A COMPASSIONATE
AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG
(INAD) EXEMPTION FOR SALMON GONADOTROPIN-
RELEASING HORMONE ANALOGUE
(sGnRH α – OVAPLANT-L) (INAD 13-298)**

Sponsor:

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

Sponsor Signature

Date Approved

Manufacturer:

Syndel
1441 W Smith Road
Ferndale, WA 98248

Office for Coordination of sGnRH α (Ovaplant-L) INAD:

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

Proposed Starting Date: June 1, 2019

Proposed Ending Date: June 30, 2027

Study Director: Bonnie Johnson

Clinical Field Trial Location:

Facility: _____

Investigator: _____

TABLE OF CONTENTS

I. STUDY IDENTIFICATION AND TITLE.....	4
II. SPONSOR.....	4
III. INVESTIGATORS/FACILITIES.....	4
IV. PROPOSED STARTING AND COMPLETION DATES.....	4
V. BACKGROUND/PURPOSE.....	5
VI. SPECIFIC OBJECTIVES.....	6
VII. MATERIALS.....	7
VIII. EXPERIMENTAL UNIT.....	10
IX. ENTRANCE CRITERIA.....	10
X. TREATMENT GROUPS.....	11
XI. TREATMENT SCHEDULES.....	13
XII. TREATMENT RESPONSE PARAMETERS.....	14
XIII. FORMS FOR DATA COLLECTION.....	15
XIV. RECORD KEEPING PROCEDURES.....	16
XV. DISPOSITION OF INVESTIGATIONAL ANIMALS.....	16
XVI. DISPOSITION OF INVESTIGATIONAL DRUG.....	16
XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES.....	16
XVIII. PLANS FOR DATA ANALYSIS.....	18
XIX. PROTOCOL AND PROTOCOL AMENDMENTS.....	18
XX. PROTOCOL DEVIATIONS.....	18
XXI: E.O. 13891.....	18
LITERATURE CITED.....	18
APPENDIX I.....	19
APPENDIX II.....	20
APPENDIX IIIA.....	21
APPENDIX IIIB.....	22
APPENDIX IV.....	23
APPENDIX V.....	24

APPENDIX VIA.....	25
APPENDIX VIB.....	26
ALL DATA MUST BE ENTERED THROUGH THE ONLINE INAD DATABASE:.....	27
FORM OVAPLANT-L-W.....	28
FORM OVAPLANT-L-1.....	30
FORM OVAPLANT-L-2.....	31
FORM OVAPLANT-L-3.....	32
FORM OVAPLANT-L-4N.....	35

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR SALMON GONADOTROPIN-RELEASING HORMONE ANALOGUE (sGnRHa – Ovaplant-L) UNDER INAD #13-298

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of sGnRHa (Ovaplant- L) liquid gel injectable to induce gamete maturation (ovulation and spermiation) in a variety of fish species under INAD 13-298.

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: Syndel
1441 W Smith Road
Ferndale, WA USA 98248
Email: info@syndel.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: June 1, 2019

Proposed Completion Date: June 30, 2027

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. While males may produce milt, the quantity and quality is often greatly reduced in captivity (reviewed in Zohar and Mylonas, 2001). 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.

In males, reproductive dysfunction is most often exhibited by a reduction in milt production and quality. While these reproductive dysfunctions may be addressed through environmental manipulations (i.e. temperature, photo period), 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 LHRHa), provide a safe, effective approach to overcome these physiological dysfunction(s) and enhance sexual maturity and spawning of male and 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 (and GnRHa) have structural similarities across many teleost fish species, and thus the use of GnRHa such as “Ovaplant -L” can be successfully applied with success to a wide range of teleost fish species.

Further, in order to maintain the health of both wildstock and domestic brood fish, it is beneficial to minimize overall fish handling. During normal spawning at a hatchery, it may be necessary to handle and examine individual fish weekly over a prolonged period of time. Such procedures can be extremely stressful to broodstock and result in severely compromised fish health and potential fecundity. A sustained release hormone, such as “Ovaplant-L”, can reduce handling requirements to a single hormone administration event, followed by predictable gamete collection timing; this will result in reduced fish handling and lowering anthropogenic-driven handling stress.

B. Purpose of INAD:

The purpose of this INAD for sGnRHa in a sustained release gel (“Ovaplant-L”) for injection is to develop clinical field trial data that will be used to determine the efficacy and appropriate treatment regimens for inducing ovulation and/or spermiation in a variety of cultured and wildstock fish species. These data will be used to support a new animal drug application (NADA) for “Ovaplant-L” (sGnRHa sustained release injection).

The U. S. Fish and Wildlife Service (USFWS) and Syndel anticipates that it may take several years to complete all the technical section data for a NADA for “Ovaplant-L”. We are aware that opportunities for the use of “Ovaplant-L” in wild and cultured fish species will be unpredictable, and that there is no way of knowing in advance if, when, or where the opportunities for pivotal studies will be encountered. The USFWS and Syndel believes it is likely that data from 5-7 treatment seasons will be required in order to adequately assess the efficacy of “Ovaplant-L” (sGnRHa sustained release injection) treatment on induction of 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 (Ovaplant- L) on gamete maturation in both cultured fish under typical hatchery situations and on critical wildstock species
2. Provide the opportunity for fish culturists and fisheries managers to legally use sGnRHa (Ovaplant-L) 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 (Ovaplant-L) use in fish. Specifically, sGnRHa (Ovaplant-L) 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 analog
(sGnRHa)

Product Name: Ovaplant-L®

Product Code: 13460

Chemical Name: [Des-Gly¹⁰, D-Arg⁶, Trp⁷, Leu⁸] - LHRH, ethyl

amide CAS Number: None

Amino Acid Profile: pGlu-His-Trp-Ser-Tyr-D-Arg-Trp-Leu-Pro-NHC₂H₅

Appearance: Viscous liquid, off white to cream colored suspension

b. Strength and dosage form

sGnRHa ("Ovaplant-L") is a synthetic peptide analogue of salmon gonadotropin releasing hormone (sGnRHa). It is presented in a sucrose-gel based matrix for either an intramuscular or intracoelomic injection. "Ovaplant-L" is available at a concentration of 100µg/mL. Based on diffusion kinetic studies performed at 10C by the manufacturer, the eMax value (predicted maximum drug release) is 97% and the ET50 value (time for half the sGnRHa to be released) is 75 hours. This follows a consistent, simple diffusion profile.

c. Manufacturer, source of supply

Syndel, USA
1441 W Smith Road
Ferndale, WA 98248

Contact Person: Jason Montgomery
Phone: 800-283-5292
Fax: 360-384-0270
email: jasonm@syndel.com

Website: www.syndel.com

2. Verification of Drug Integrity/Strength:

The Manufacturer will provide the analytical data necessary to establish the purity of each lot of sGnRHa (Ovaplant-L) supplied. The lot number and date of manufacture for each batch of sGnRHa (Ovaplant-L) 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 Ovaplant-L-1) will clearly identify the lot number for all sGnRHa shipments. If the integrity of the sGnRHa (Ovaplant-L) 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 Ovaplant-L-2). All un-usable sGnRHa (Ovaplant-L) must be destroyed by following the disposal methods described in the SDS.

3. Storage Conditions

sGnRHa (Ovaplant-L) 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 ($\sim 4^{\circ}\text{C}$) and out of direct sunlight. 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 (Ovaplant-L) 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 sGnRHa (Ovaplant-L; see Appendix IV). Each person involved with the study and each person who may be present during the use of sGnRHa (Ovaplant-L) shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with sGnRHa (Ovaplant-L).

5. Investigational Labeling

A copy of the label to be attached to each container of sGnRHa (Ovaplant-L) is provided in Appendix V. Although investigational labels will be affixed to containers by the manufacturer, it is the responsibility of the Investigator to ensure proper labeling of all containers of sGnRHa (Ovaplant-L).

6. Accountability

Syndel will be the sole supplier of sGnRHa (Ovaplant-L) 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 sGnRHa (Ovaplant- L):

Immediately upon receiving an order/shipment of sGnRHa (Ovaplant-L), the Investigator will complete Form Ovaplant-L-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 MT-1s are received by the Study Director within 10 days of drug receipt.

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

At the conclusion of field trials, all remaining sGnRHa (Ovaplant-L) will be disposed of by following the disposal methods in the Safety Data Sheet (note: unless sGnRHa (Ovaplant-L) is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all sGnRHa (Ovaplant-L) must be properly recorded and accounted for on the Chemical Use Log (Form Ovaplant-L-2). The Study Monitor will be responsible for verifying the quantity of sGnRHa (Ovaplant-L) remaining on hand versus the amount indicated on Form Ovaplant-L-2. **Note:** sGnRHa (Ovaplant-L) can be transferred to other facilities that are participating under INAD 13-298. Transfers must be shown in the Drug Inventory section of the database (formerly Form Ovaplant-L-2).

7. Preparation Procedures

sGnRHa (Ovaplant-L) is formulated to provide a sterile, liquid injectable in a multi-use container. Ovaplant Liquid is available at a concentration of 100ug/mL per mL solution.

Recommended dose of 10ug/kg as single injection is sufficient to induce maturation in most species. Administer to fish as an intraperitoneal (IP) or intramuscular (IM) injection using a sterile syringe and needle (18 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 g needle recommended). 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 sGnRHa (Ovaplant-L) INAD will need to complete several forms located in the online INAD database. 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 sGnRHa (Ovaplant-L) 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

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

D. Environmental conditions

Since sGnRHa (Ovaplant-L) will be injected directly into the musculature or peritoneum, there will be no drug discharge from participating facilities. Therefore, sGnRHa (Ovaplant-L) qualifies for a categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.33(e).

Drug discharge must be in compliance with local **NPDES** permitting requirements.

Environmental conditions will be variable and include a broad spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on Form Ovaplant-L-3.

- 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 Ovaplant-L-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 Ovaplant-L-2 and Ovaplant-L-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 sGnRHa (Ovaplant-L) treatment. In some cases, particularly with respect to wildstock populations, the number of broodfish available at a given time for sGnRHa (Ovaplant-L) 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:

<u>Treatment Group Size</u>	<u>Control Group Size¹</u>
0 - 10 fish	0 (too few fish for data analysis)
11 - 30 fish	5 fish
31 - 50 fish	10 fish
51 - 75 fish	15 fish
76 -100 fish	20 fish
101-300 fish	25 fish
>300 fish	30 fish

¹ Minimum number of fish per control group

- 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 sGnRH α (Ovaplant-L)**. 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

sGnRHa (Ovaplant-L) should be brought to room temperature and injected into the dorsal musculature or peritoneum using an appropriately sized sterile needle and syringe (18g). Injections should be administered into the musculature immediately anterior and lateral (on either side) to the dorsal fin. Insert the sGnRHa (Ovaplant-L) needle, while holding the fish firmly and inject into the dorsal musculature. 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 10-75 ug sGnRHa/kg body weight. Although certain situations involving very small broodfish (e.g. fish less than 1 kg body weight) may require a higher dosage rate, dosage will never exceed 150 ug sGnRHa/kg body weight. Note: the recommended dose of 10ug/kg as single injection is sufficient to induce maturation in most species. The dose will be dependent on the species so you can contact Syndel for more information on what dose may work for your fish.

C. Dosing interval and repetition

sGnRHa (Ovaplant-L) will be administered as single treatment event only.

D. Drug preparation procedures

sGnRHa (Ovaplant-L) will be supplied by Syndel as a sterile, liquid injectable in a multi-use container. Ovaplant Liquid is available at a concentration of 100ug/mL per mL 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 sGnRHa (Ovaplant-L) 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 (Ovaplant-L) treatment involved should be appropriately labeled.

An exception to this concomitant therapy is anesthetics may be used to sedate fish prior to sGnRHa (Ovaplant-L) treatment. If an anesthetic is used please note which one was used in Form Ovaplant-L-3 under the description of results section.

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

1) Primary Parameters

The primary response parameter for evaluating the effect of sGnRHa (Ovaplant-L) 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 Mortality 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 sGnRHa

(Ovaplant-L) 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. Investigators should carefully observe all treated fish for any signs of adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions on Form Ovaplant-L-3. **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 Ovaplant-L-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 (Ovaplant-L) INAD will need to complete the following forms:

Form Ovaplant-L-W. Worksheet for Designing Clinical Field Trials under INAD 13-298 - located in the New Study Request tab

Form Ovaplant-L-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 Ovaplant-L-2. Drug Inventory Form for use of sGnRHa (Ovaplant-L) under INAD 13-298 - located in the Manage/View Drug Inventory tab and filled out in Form Ovaplant-L- 3 to show use

Form Ovaplant-L-3. Results Report Form for use of sGnRHa (Ovaplant-L) under INAD 13-298 - located in the Active Studies table on the home page

Form Ovaplant-L-4N. Necropsy Report - located in Form Ovaplant-L- 3

Copies of these forms are attached to this Study Protocol and are to be used as a guide for collecting the data that will be entered into the online INAD database. **Actual reporting is accomplished on forms located on 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. **All fish treated with sGnRH α (Ovaplant-L) must be maintained in culture facilities indefinitely or destroyed.** As drug release/residue data from sGnRH α (Ovaplant-L) are inconclusive, treated fish will not be allowed to be released/stocked or to enter the food chain. The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form Ovaplant-L-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

sGnRH α (Ovaplant-L) will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or outdated sGnRH α (Ovaplant-L) 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 sGnRH α (Ovaplant-L) must be properly recorded and accounted for on the Chemical Use Log (Form Ovaplant-L-2). The Study Monitor will be responsible for verifying the quantity of sGnRH α (Ovaplant-L) remaining on hand versus the amount indicated on Form Ovaplant-L-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 sGnRH α (Ovaplant-L) 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 sGnRHa (Ovaplant-L). 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 sGnRHa (Ovaplant- L) 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

sGnRHa (Ovaplant- L) will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). sGnRHa (Ovaplant-L) 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 Ovaplant-L-W, Ovaplant-L-1, Ovaplant-L-2, Ovaplant-L-3, and Ovaplant-L- 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 signed and dated. Deviation statements should be documented on Form Ovaplant-L-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 sGnRHa (Ovaplant-L) INAD #13-298

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

1

Appendix II. Study Monitors for sGnRHa (Ovaplant-L) INAD #13-298

Note: This information will be provided directly to CVM

1Appendix IIIa. Facilities and Names of Investigators Participating under sGnRHa (Ovaplant-L) INAD #13-298

Note: This information will be provided directly to CVM and Syndel

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 sGnRHa (Ovaplant-L) INAD #13-298

The SDS for LHRHa Liquid/LHRHa can be found at the drug sponsor's website: SDS:
https://syndel.com/wp-content/uploads/2020/01/SDS_Ovaplant-L_30Nov2018.pdf

1

Appendix V. Investigational Label for sGnRHa (Ovaplant-L) INAD #13-298

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."

1

Appendix VIa. Fish Species Treated under sGnRHa (Ovaplant-L) INAD #13-298

All finfish

1

Appendix VIb. Table of Facilities and Fish Stocks Treated under sGnRHa (Ovaplant-L) INAD #13-298

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.

1 Form Ovaplant-L-W: Worksheet for Designing Clinical Field Trials under sGnRHa INAD 13-298

INSTRUCTIONS

1. Investigator must fill out Form Ovaplant-L-W for each trial conducted under this INAD **before** actual use of salmon Gonadotropin Releasing Hormone analog.
2. Investigator should forward a copy of Ovaplant-L-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 males					Number of treated females	
Number of control males					Number of control females	
Anticipated date of treatment					Estimated total amount of drug for proposed treatments (mL)	
Intended sGnRHa dosage (ug/kg)	Females		Males		Method of administration	Injection (IM or IP)
					Number of injections per fish	single
Drug manufacturer	Syndel USA, Inc.				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):

Fish treated with sGnRHa (Ovaplant-L) may not be stocked, released, or harvested for human consumption. All treated fish must ultimately be destroyed. 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 the Safety Data Sheet for salmon gonadotropin releasing hormone analog (Ovaplant-L) and have been provided protective equipment, in good working condition, as described in the SDS.

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

1Form Ovaplant-L-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 Ovaplant-L-1 **immediately** upon receipt of sGnRH α .
2. Investigator should forward a copy of Form Ovaplant-L-1 to the Study Director at the AADAP Office

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

Name of Drug	sGnRH α (Ovaplant-L)	INAD Number	13-298
Proposed Use of Drug	To induce gamete maturation in a variety of fish species.		
Date of CVM Authorization Letter	To be determined		
Source of Drug	Syndel USA		
Date of Drug Receipt		Amount of Drug Received (mL)	
Drug Lot Number		Study Worksheet Number	
Name of Investigator			
Address of Investigator			
Location of Trial			
Approximate Number of Treated Animals			
Study Protocol Number	13-298		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum total dose	150 ug/Kg body weight		
Methods of Administration	Injection (IM or IP)		
Withdrawal Period	No release of fish treated with sGnRH α (Ovaplant-L)		
Date Prepared:		Investigator:	
Date Reviewed:		Study Monitor:	
Date Reviewed:		Sponsor:	

1Form Ovaplant-L-2: Drug Inventory Form

For Use in sGnRHa (Ovaplant-L) Clinical Field Trials Conducted under sGnRHa INAD 13-298

INSTRUCTIONS

1. Investigator should initiate a new form Ovaplant-L-2 **immediately** upon receipt of each shipment of salmon gonadotropin releasing hormone analog.
2. Each lot number of sGnRHa (Ovaplant-L) may be used for multiple treatment regimens.

Qty of sGnRHa from

Reporting

previous page (mL) _____ Facility _____ individual _____

Date	Amount of new sGnRHa received (mL)	Lot number of sGnRHa received	Study Number	Amount of sGnRHa used in treatment (mL)	sGnRHa transferred (mL)	sGnRHa discarded (mL)	sGnRHa remaining on hand (mL)	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						

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

1STUDY NUMBER _____

Form Ovaplant-L-3: Results Report Form

For Use in sGnRHa (Ovaplant-L) Clinical Field Trials Conducted under sGnRHa
INAD 13-298

INSTRUCTIONS

- Investigator must fill out Form Ovaplant- L-3 no later than **10 days** after completion of the study period. Attach lab reports and other information.
- If sGnRHa (Ovaplant-L) was not used under the assigned Study Number, contact the Study Director at the AADAP Office to close-out the study.
- Investigator should forward a copy of Form Ovaplant-L-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 (mL)	
Fish species treated		Water temperature (°F)	
Drug dosage - males (ug/kg body wt)		Drug dosage - females (ug/kg body wt)	
Average fish weight (gm)		Average fish length (in)	
Number of treated males		Number of treated females	
Number of control males		Number of control females	
Treatment date(s)			
Treatment method (IP or IM Injection)			
Number of injections per male	Single	Number of injections per female	Single

Spawning/evaluation interval (time from treatment until spawning)		Spawning/evaluation date(s)	
--	--	-----------------------------	--

Ovaplant-L Results Record

INSTRUCTIONS

1. "Ripe" females are those fish that have ovulated or released their eggs. "None-ripe" fish are the converse.
2. Motility Score based on a scale of 0-4 (see Study Protocol Section XII).
3. Use Additional copies of this form for additional treatment days

Be sure the facility name is written here: _____

sGnRHa TREATED FISH - Females					CONTROL FISH - Females					
Date Treated	Date Evaluated	Ripe	Non-ripe	% Eye-up	% Hatch	Date Evaluated	Ripe	Non-ripe	% Eye-up	% Hatch

sGnRHa TREATED FISH - Males					CONTROL FISH - Males					
Date Treated	Date Evaluated	Ripe	Non-ripe	Milt/fish (ml)	Motility Score	Date Evaluated	Ripe	Non-ripe	Milt/fish (ml)	Motility Score

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:

Observed
withdrawal
period :

Fish treated with sGnRHa (Ovaplant-L) may not be stocked, released, or harvested for human consumption. All treated fish must ultimately be destroyed. Investigator should initial here to indicate compliance with disposition requirements of sGnRHa (Ovaplant-L) treated fish

_____ **NEGATIVE REPORT** Salmon gonadotropin releasing hormone analog (Ovaplant-L) 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: _____

1Study Number: _____

Page 1 of 1

Form Ovaplant-L-4N: Necropsy Report Form

For Use in sGnRH α (Ovaplant-L) Clinical Field Trials Conducted under INAD 13-298

INSTRUCTIONS

1. Investigator must fill out Form Ovaplant-L-4N for all fish that die or are euthanized during the study period. Use a new copy of Form Ovaplant-L-4N for each individual fish.
2. Submit all Form Ovaplant-L-4Ns with appropriate Form sGnRH α /Ovaplant-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 ☐ exophthalmia _____

stomach _____

body cavity _____

gastrointestinal tract _____

gall bladder _____

gas bladder

adipose tissue _____

musculature _____

implant site _____

other _____

Investigator: _____ Date: _____

NOTICES

Paperwork Reduction Act

In accordance with the Paperwork Reduction Act (44 U.S.C. 3501 *et seq.*), the U.S. Fish and Wildlife Service collects information necessary to permit the use of an investigational new animal drug to generate data to support a new animal drug approval (NADA) as part of the Fish and Aquatic Conservation fish health network. Your response is voluntary, but is required to obtain or retain a benefit. According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. OMB has approved this collection of information and assigned Control No. 1018-####.

ESTIMATED BURDEN STATEMENT

We estimate public reporting for this collection of information to average 4 hours, including time for reviewing instructions, gathering and maintaining data, and completing and reviewing the form. Direct comments regarding the burden estimate or any other aspect of the form to the Service Information Clearance Officer, Fish and Wildlife Service, U.S. Department of the Interior, 5275 Leesburg Pike, MS: PRB (JAO/3W), Falls Church, VA 22041-3803, or via email at Info_Coll@fws.gov. Please do not send your completed form to this address.

FREEDOM OF INFORMATION ACT STATEMENT

Information provided to the Service is generally subject to release to the public under the Freedom of Information Act (FOIA). Certain information, however, may be subject to withholding if the Service determines that the information is a trade secret and/or commercial or financial information that is privileged or confidential. To the extent you are submitting business information that falls into one of these categories, you must clearly mark this information as "Business Confidential" in order for the Service to assess the applicability of FOIA Exemption 4. Any information provided by you that is not marked as "Business Confidential" will be considered releasable to the public under the FOIA [43 CFR 2.26 – 2.33].