[1]STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR THE USE OF 17-ALPHA METHYLTESTOSTERONE IN TILAPIA (INAD #11-236)

Sponsor:

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

_____ _____

Sponsor Signature Date Approved

Manufacturer (medicated feed):

Rangen Inc.
P.O. Box 706
Buhl, ID 83316

Office for Coordination of 17-ALPHA METHYLTESTOSTERONE INAD:

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

Proposed Starting Date	October 1, 2007
Proposed Ending Date	December 31, 2026
Study Director	Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility: _____

Investigator: _____

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STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR THE USE OF 17-ALPHA METHYLTESTOSTERONE IN TILAPIA UNDER INAD #11-236

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of 17-alpha methyltestosterone when administered in feed to early life stage tilapia to produce populations comprised of greater than 90% male fish. Clinical field trials will be conducted on early life stage tilapia held at a various tilapia production facilities under a variety of environmental conditions under INAD #11-236.

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 (medicated feed):

Rangen Inc.
P.O. Box 706
Buhl, ID 83316

Contact Person at Rangen Inc.:

David Brock
P.O. Box 706
Buhl, ID 83316
Ph. 1-800-657-6446 x 3332

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. It is important to note that all facilities need to be approved by the FDA/CVM Environmental Team prior to participation in the INAD. If any of the approved conditions (i.e., number of treated fish; number of treatments each year; discharge of treated water; etc…) changes at your facility then a new approval will be required.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: October 1, 2007

Proposed Completion Date: December 31, 2026

V. BACKGROUND/PURPOSE

A. Background Information:

Larval stages of many teleost species contain both ovarian and testicular tissue, and sexual differentiation commences shortly after hatching or after the initiation of feeding (Yamamoto, 1969; Donaldson and Hunter, 1982; Yamazaki, 1983). Various techniques have been developed for the control of sexual differentiation in a variety of fish species (Donaldson and Hunter, 1982). These techniques have typically involved the use of either androgen or estrogen treatment to override the endogenous mechanisms of sex determination in developing larval stages and direct sexual differentiation toward the production of either males or females. Treatment regimens have generally involved immersion of larval stages in water containing a steroid, incorporation of a steroid in the larval diet, or both. As numerous factors such as dosage, timing, duration, and environmental conditions often influence efficacy, results have been somewhat variable.

Although the gonadal tissue of tilapia remains undifferentiated at hatch, tilapia generally attain sexual maturity by three to six months of age, and begin to immediately reproduce. This somewhat precocious reproduction is the number one impediment to the development of successful strategies for the commercial production of tilapia. In response to this problematic issue, strategies for monosex male culture have been evaluated including: 1) manual separation of the sexes by visual examination; 2) hybridization; and 3) sex reversal/direction via steroid administration.

1. Manual separation of the sexes via visual examination (or hand sexing) has been found to be a tedious and time consuming process, prone to significant human error, and less productive than other methods. Currently, hand sexing of tilapia is practiced by only a limited number of fish farmers in underdeveloped countries.

2. Hybridization involves the crossing of two different species of tilapia that can result in the production of 95-100% male offspring. The most frequently used crosses suitable for aquaculture production are *Tilapia nilotica* (F) x *T. hornorum* (M); *T. mossambica* (F) x *T. hornorum* (M); and *T. nilotica* (F) x *T. aurea* (M). However, there are limitations to hybridization that include behavioral incompatibilities between the two species that lead to significantly decreased fingerling production; the high potential for the contamination of the broodfish

populations; and the high costs associated with the sophisticated equipment and techniques necessary to ensure and confirm broodfish purity. Hybridization is currently practiced on only a limited basis worldwide.

3. Gonadal differentiation in tilapia typically occurs between 8 to 25 days post-hatch, dependent upon environmental conditions. It has been demonstrated that the oral administration of the synthetic androgen 17-alpha methyltestosterone to newly hatched tilapia fry (3-12 days old) for ~28 consecutive days results in populations comprised of greater than 90% males (Green et al., 1997; Rani and Macintosh, 1997; and Teichert-Coddington et al., 2000). The excess androgen that is introduced into the early life stage fish overrides endogenous hormones and directs sexual differentiation towards the formation of testis. The use of orally administered 17-alpha Methyltestosterone has been shown to be an efficacious, cost-effective, and efficient methodology to produce populations of male tilapia.

Of the three above-described methods/strategies for the monosex culture of male tilapia, oral administration of 17-alpha methyltestosterone is best suited for successful tilapia production in the United States.

B. Purpose of INAD:

The purpose of this basic INAD for 17-alpha methyltestosterone is to develop clinical field trial data that will be used to determine the efficacy of 17-alpha methyltestosterone when administered in feed to early life stage tilapia to produce greater than 90% male fish. These data will be used to support a new animal drug application (NADA) for 17-alpha methyltestosterone.

The U. S. Fish and Wildlife Service (USFWS) anticipates that data from multiple treatment seasons will be required in order to adequately assess the efficacy of 17-alpha methyltestosterone medicated feed treatment to produce greater than 90% male fish.

VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the effectiveness of 17-alpha methyltestosterone to effect sex reversal in early life stage tilapia under a variety of environmental conditions (e.g. temperature, water hardness, pH, turbidity, etc).

2. Provide an opportunity for fish culturists and fisheries managers to legally use 17-alpha methyltestosterone to maintain and manage production stocks of fish during the period of time necessary for collection of efficacy, safety, and residue data needed to support a NADA for 17-alpha methyltestosterone use in tilapia.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

   a. Active ingredient

Common Name:	17-alpha methyltestosterone
Chemical Name:	17beta-hydroxy-17-methylandrost-4-ene-3-one
Chemical Formula:	$C_{20}H_{30}O_2$
Chemical Family:	Steroid
C.A.S. Registry No.:	58-18–4
Grade:	USP
Form:	white crystalline powder
Solubility in water:	insoluble
Melting point:	162^{o}C
Odor:	no information available

   b. Supplier of 17-alpha methyltestosterone

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

Only the authorized feed manufacturer (i.e. Rangen Inc.) will be allowed to purchase, receive, and store 17-alpha methyltestosterone from Syndel. The feed manufacturer will comply with FDA Good Manufacturing Practices. The feed manufacturer will also ensure that the pure drug is stored in accordance with all federal regulations that apply to Schedule III controlled substances.

2. Verification of Drug Integrity/Strength:

The feed manufacturer (Rangen Inc.) will provide the analytical data necessary to establish the purity of each lot/batch of 17-alpha methyltestosterone medicated feed supplied to investigators. The batch number and date of manufacture for each batch of 17-alpha methyltestosterone medicated feed will be placed on the label of each bag/container of feed. The form *Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals* (Form MT-1) will clearly identify the batch number and date of manufacture of 17-alpha methyltestosterone medicated feed shipments. If the integrity of the 17-alpha methyltestosterone medicated feed 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 MT-2). All un-usable 17-alpha methyltestosterone medicated

feed must be destroyed by incineration. The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

17-alpha methyltestosterone medicated feed will be stored in the original container supplied by the feed manufacturer with the appropriate investigational label attached. Medicated feed should be stored in a cool, dry, well ventilated area away from direct sunlight and sources of heat or flame. Exercise due caution to prevent damage to, or leakage from, the container. Medicated feed should be stored at temperatures, and for periods of time, not to exceed guidelines set by the feed manufacturer.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Safety Data Sheet (SDS) for 17-alpha methyltestosterone (Appendix IV). Each person involved with the study and each person who may be present during the administration of 17-alpha methyltestosterone medicated feed shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with 17-alpha methyltestosterone medicated feed. Eye and skin contact should be avoided at all times. All handlers of medicated feed will be provided with personal protective equipment and given training with respect to the proper storage, handling, and administration of 17-alpha methyltestosterone medicated feed. No special respiratory protection is required during normal application.

5. Investigational Labeling

A copy of the label to be attached to each container of 17-alpha methyltestosterone medicated feed is provided in Appendix V. Although investigational labels will be affixed to medicated feed containers by the feed manufacturer, it is the responsibility of the Investigator to ensure proper labeling of all containers of 17-alpha methyltestosterone medicated feed.

6. Accountability

Syndel will be the only entity authorized to purchase 17-alpha methyltestosterone; Syndel and Rangen, Inc. will be the suppliers able to receive, and store 17-alpha methyltestosterone; and Rangen will be the sole supplier of 17-alpha methyltestosterone medicated feed 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 17-alpha methyltestosterone medicated feed:

Immediately upon receiving an order/shipment of 17-alpha methyltestosterone medicated feed, the Investigator must complete Form MT-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 17-alpha methyltestosterone medicated feed on-hand. A Chemical Use Log (Form MT-2) must be completed and maintained by each Investigator. Each time 17-alpha methyltestosterone medicated feed is used, it must be recorded by the Investigator in the Results Report form in the “Amount Of Drug Used” table and in the “MT Medicated Daily Feeding Record”.

At the conclusion of field trials, all remaining 17-alpha methyltestosterone medicated feed will be destroyed by incineration (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 17-alpha methyltestosterone medicated feed must be properly recorded and accounted for on the Chemical Use Log (Form MT-2). The Study Monitor will be responsible for verifying the quantity of 17-alpha methyltestosterone medicated feed remaining on hand versus the amount indicated on Form MT-2. **Note:** 17-alpha methyltestosterone medicated feed can be transferred to other facilities that are participating under INAD 11-236. Transfers must be shown in the Drug Inventory section of the database (formerly Form MT-2).

7. Preparation Procedures

There are no special preparation procedures for 17-alpha methyltestosterone medicated feed as all feed preparation will be conducted by the authorized feed manufacturer. However, investigators will be required to administer the medicated feed to the fish at the prescribed dosage, which will require accurate weighing of both fish and feed.

B. Items Needed for Treatment, Sample Collection, Observations, Etc.:

Treatment and diagnostic equipment should include a balance, thermometer, dissolved oxygen meter, dip nets, 10% formalin, and nalgene sample bottles.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the 17-alpha methyltestosterone medicated feed 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 will consist of a contained or isolated group of fish. This will generally be a group of fish contained in a tank, raceway, or pond. In some cases, the experimental unit may be individual animals.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

It is important to note that all facilities need to be approved by the FDA/CVM Environmental Team prior to participation in the INAD. If any of the approved conditions (i.e., number of treated fish; number of treatments each year; discharge of treated water; etc…) changes at your facility then a new approval will be required before new treatments can begin. The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol for the current calendar year before 17-alpha methyltestosterone medicated feed can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (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, number, etc.) is presented in Appendix VIb.

C. Environmental conditions

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

D. 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 MT-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 MT-2 and MT -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, or it may be individual animals.

B. Non-treated control groups will not be a requirement for clinical field trials evaluating the efficacy of 17-alpha methyltestosterone medicated feed conducted under this study protocol for INAD 11-236.

C. Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. **Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals.** Assignment to control and treatment groups should be random and designed to avoid bias. It is important that all test fish are treated/handled 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 help to 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 17-alpha methyltestosterone medicated feed. 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

17-alpha methyltestosterone will be administered only as a medicated feed treatment. Rangen Inc. will be the only source of 17-alpha methyltestosterone medicated feed used under this INAD.

B. Dose to be administered

17-alpha methyltestosterone (MT) will be administered at a dosage of 9 mg MT/kg of fish biomass. 17-alpha methyltestosterone will be incorporated by the manufacturer into standard tilapia feed at a rate of 60 mg MT/kg feed. Based on standardized industry procedures for tilapia production, newly hatched tilapia fry should be fed at a rate of 15% body weight per day. Hence, tilapia fry should be fed 150 g MT-medicated feed per kg of fish biomass daily under this INAD.

C. Dosing interval and repetition

17-alpha methyltestosterone medicated feed will be administered as a single treatment regimen, with no repetition of treatment.

D. Duration of treatment

17-alpha methyltestosterone medicated feed will be fed to tilapia fry for 28 consecutive days. Application of medicated feed will be permanently terminated upon completion of the 28-day treatment period. **No re-treatment of fish will be allowed**.

E. Detailed procedures for drug administration

Standard personal protective equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when administering 17-alpha methyltestosterone medicated feed. Medicated feed for each individual lot of fish should be accurately weighed prior to treatment. Fish should be fed in such a manner as to ensure optimal consumption of 17-alpha methyltestosterone medicated feed.

F. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be 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 minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with 17-alpha methyltestosterone medicated feed.

An exception to this concomitant therapy is that formalin or diquat treatments are acceptable to use when 17-alpha methyltestosterone medicated feed treatments are in progress **provided the longest of the withdrawal periods is observed**. Formalin must be used under the conditions of the approved label and diquat must be used under the conditions of the INAD protocol. If formalin and/or diquat is used please note which one was used in Form MT-3 under the description of results section.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat fish based on hatchery records or hatchery management practices that indicate 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 MT-3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

The efficacy of 17-alpha methyltestosterone medicated feed to produce tilapia populations comprised of greater than 90% male fish will be the primary response variable of clinical field efficacy trials. The sex of individual fish will be determined by evaluation of gonadal tissue according to procedures of the gonadal squash technique as described by Guerrero and Shelton, 1974. Gonads will be classified as testis, ovary, or ovotestis (gonads containing both ovarian and testicular tissue). However, as the proposed claim is for the production of male fish, identification of a gonad as either ovary or ovotestis will be considered a treatment failure.

Pivotal studies have been completed and accepted for the Efficacy and Target Animal Safety technical sections. Because these studies have been accepted, fish will no longer be required to be sent to the AADAP Office or evaluated for efficacy. However, if facilities would like to evaluate 60 fish from treatments to determine the sex ratio of a population, this can still be reported in Form MT-3. Fish sampled for determination of sex ratio must be a minimum of 5 cm in total length (i.e. ~60 days post-hatch) and should be collected randomly from the treatment lot by the Investigator.

2. Secondary Parameters

Secondary parameters include general observations on fish behavior and response to routine culture activities. Secondary parameters of interest include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, mortality, etc.

As a result of the potential diversity of treatment circumstances involved in these studies, Investigators are encouraged to provide copies of their own daily lot observation forms for individual rearing units. Investigators may also choose to create their own forms for purposes of recording source data under this INAD. **All supplementary data forms should include the Study Number and be attached to Form MT-3.**

3. Adverse Reactions

Any adverse reaction to treatment should be reported **immediately** to the Study Monitor, who will in turn notify the Study Director. Such responses might include changes in water quality, extremely negative responses/behavior by fish, or hazards to the applicator. Although 17-alpha methyltestosterone medicated feed has been used fairly extensively in the culture of tilapia, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific 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 MT-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.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol for 17-alpha methyltestosterone medicated feed has been approved and treatments are scheduled, the Investigator at each facility covered by the INAD will need to complete the following forms:

Form MT-W. Worksheet for Designing Individual Field Trials under MT INAD 11-236 - located in the New Study Request tab

Form MT-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 MT-2. Chemical Use Log for Clinical Field Trials Using MT Medicated Feed Under INAD 11-236 – located in the Manage/View Drug Inventory tab and filled out in Form MT-3 to show use

Form MT-2a. Daily Record of MT Medicated Feed Use – located in Form MT-3

Form MT-3. Results Report Form for Clinical Field Trials Using MT Medicated Feed Under INAD 11-236 – located in the Active Studies table on the home page

Form MT-4 Is no longer needed

Form MT-5 Transfer Record of MT-treated Fingerling Tilapia Under MT INAD 11-236 – will be uploaded in Form MT-3 or emailed to the AADAP Office

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

The investigational withdrawal period for tilapia administered 17-alpha methyltestosterone medicated feed will be dependent upon fish culture practices at (or within) individual facilities. Under this INAD, two (2) separate fish culture practices are identified and defined. Investigational withdrawal period will be dependent upon which type of culture practice is utilized for individual treated lots. Culture practice definitions and respective investigational withdrawal times are as follows:

Batch Culture

Batch culture is defined as when all fish in a group/lot enter and leave the lot at the same time (sometimes referred to as "all in/all out" culture). In batch culture a defined group of similar age/size fish are stocked simultaneously for a prescribed grow-out period, which is followed by a complete harvest of the production unit. **The investigational withdrawal period for fish reared under a batch culture regimen will be 120 days.** This withdrawal period is determined based on the last day of treatment with 17-alpha methyltestosterone medicated feed.

Partial Harvest/Restock Culture

Partial harvest/restock culture is defined as the mixing of different lots of fish during the grow-out period, and the selective harvest of fish from the production unit at various times. **The investigational withdrawal period for fish reared under partial harvest/restock culture will be until such time that harvested fish reach an individual minimum weight of 350 g.**

Animals that die during treatment should be disposed of by burial or incineration. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

The Investigator must record the culture method used and verify compliance with established withdrawal period(s) of all treated fish on Form MT-3.

If MT-treated fingerling tilapia are transferred/sold to other producers for grow-out of fish to market size, the Investigator must fill out Form MT-5 and submit this form to the AADAP Office. The purpose of this form is to formally establish that all purchasers of MT-treated fingerling tilapia are aware of, and agree to comply with, the FDA-mandated withdrawal period(s) that must be observed before MT-treated tilapia may be slaughtered for processing or released for possible human consumption.

Formalin or diquat treatments are acceptable to use when 17-alpha methyltestosterone medicated feed treatments are in progress **provided the longest of the withdrawal periods is observed**.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG (i.e. medicated feed)

17-alpha methyltestosterone medicated feed will be used only in the manner and by the individuals specified in the Study Protocol. At the conclusion of field trials, all remaining 17-

alpha methyltestosterone medicated feed will be destroyed by incineration. Disposition of all 17-alpha methyltestosterone medicated feed must be properly recorded and accounted for on the Chemical Use Log (Form MT-2). The Study Monitor will be responsible for verifying the quantity of 17-alpha methyltestosterone medicated feed remaining on hand versus the amount indicated on Form MT-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 medicated feed 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 protocol 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 17-alpha methyltestosterone medicated feed under this INAD. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. The Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the 17-alpha methyltestosterone medicated feed itself) are already available at each participating facility. The use of various drugs, chemicals, and therapeutants to meet management and/or production goals is a common occurrence at most fish hatcheries. Fish hatchery managers and fisheries managers (i.e., Investigators) are well trained and well equipped to supervise these procedures (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See protocol Section VII.B. Items needed for treatment, sample collection, observations, etc.).

D. Administrator of the drug

17-alpha methyltestosterone medicated feed will be administered directly by the assigned Investigator (fish hatchery manager or fisheries manager) or under the Investigator's direct supervision (see Appendix IIIa for names). 17-alpha methyltestosterone medicated feed 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: Form MT-W, Form MT-1, Form MT-2, Form MT-2a, Form MT-3, and Form MT-5.

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 Monitor 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 summary reports 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 protocol 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 a field trials 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 out 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.** Deviations should be documented on Form MT-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

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Yamazaki, F. 1983. Sex control and manipulation in fish. Aquaculture, 33:329-354

1Appendix I. Sponsor Contact Information for 17-alpha methyltestosterone INAD #11-236

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

1Appendix II. Study Monitors for 17-alpha methyltestosterone INAD #11-236

Note: This information will be provided directly to CVM

1

1Appendix IIIa. Facilities and Names of Investigators Participating under 17-alpha methyltestosterone INAD #11-236

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

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.

1

Appendix IV. Safety Data Sheet (SDS) for 17-alpha methyltestosterone INAD #11-236

Contact AADAP for a copy of the Safety Data Sheet

1

Appendix V. Investigational Label for 17-alpha methyltestosterone INAD #11-236

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 17-alpha methyltestosterone INAD #11-236

Tilapia species:

Blue Tilapia (*Oreochromis niloticus*)

California Mozambique (*Oreochromis mossambica*)

Nile Tilapia (*Oreochromis niloticus*)

Tilapia Nilotica (*Oreochromis niloticus*)

[1]Appendix VIb. Table of Facilities and Fish Stocks Treated under 17-alpha methyltestosterone INAD #11-236

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 MT-W: Worksheet for Designing Individual Field Trials Under MT INAD 11-236

INSTRUCTIONS

1. Investigator must fill out Form MT-W for each trial conducted under this INAD **before** actual use of MT medicated feed.
2. Investigator should forward a copy of MT-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 the Study Number.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Manufacturer of MT medicated feed		**Rangen Inc.**	
MT medicated feed batch number		MT medicated feed manufacture date	
Treatment dosage	**9 mg/kg bw/day**	Treatment duration	**28 days**
Fish species to be treated		Number of fish to be treated	
Fish age (days post-hatch)		Average fish length (mm)	
Number of rearing units to be treated		Number of fish per treated rearing unit	
Number of control rearing units		Number of fish per control rearing unit	
Feed rate (% body weight fed per day)	**15**	Estimated total weight of fish treated (kg)	
Estimated amount of MT medicated feed needed for proposed treatment (kg)			
Anticipated date treatment will be initiated			

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):

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

USE AND DISPOSITION OF MT MEDICATED FEED (Environmental Safety Considerations):

☐ Investigator should initial here to indicate awareness that MT medicated feed usage and disposition must be in compliance with requirements described in the Study Protocol.

WORKER SAFETY CONSIDERATIONS:

☐ Investigator should initial here to indicate that all personnel handling MT medicated feed have read the Safety Data Sheet for 17-alpha methyltestosterone and have been provided personal protective equipment, in good working condition, as described in the Study Protocol.

Date Prepared: _____ **Investigator:** _____

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

FORM MT-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 MT-1 **immediately** upon receipt of 17-alpha methyltestosterone medicated feed.
2. Investigator should forward a copy of Form MT-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 the FDA:

Name of Drug	17-alpha methyltestosterone medicated feed		
INAD Number	11-236	**Study Number**	
Proposed Use of Drug	Production of tilapia populations comprised of greater than 90% male fish (i.e. sex reversal)		
Date of CVM Authorization Letter	July 23, 2020		
Date of Medicated Feed (MF) Receipt		**Amount MF Received (kg)**	
Medicated Feed (MF) Batch Number		**MF Manufacture Date**	
Location of Trial (facility name)			
Name of Investigator			
Address of Investigator			
Pivotal Study (yes/no)		**Non-pivotal Study (yes/no)**	
Approximate Number of Treated Animals		**Approximate Number of Control Animals**	
Number of Animals Used Previously[1]			
Study Protocol Number	11-236		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	9 mg/kg body weight for 28 consecutive days		
Methods(s) of Administration	Medicated feed		
Withdrawal Period	Batch culture = 120 days Partial harvest/restock culture = 350 g fish weight		

[1] To be filled out by AADAP

Date Prepared: _____ **Investigator:** _____

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

Date Reviewed: _____ **Study Director:** _____

OMB Control No. 1018-####

Expires ##/##/20##

Form MT-2. Chemical Use Log for Clinical Field Trials Using MT Medicated Feed Under INAD #11-236

Instructions: 1. Investigator should initiate a new Form MT-2 **immediately** upon receipt of each shipment of 17-alpha methyltestosterone medicated feed.

2. Each lot number of 17-alpha methyltestosterone medicated feed may be used for multiple treatment regimens.

Quantity on Hand From Previous Page (lbs): _____ **Reporting Facility:** _____ **Individual:** _____

MT Medicated Feed Batch Number and Manufacture Date	Date Received	Amount Received (kg)	Dates Used	Study Number	MT Medicated Feed Used for Treatment (kg)	MT Medicated Feed Shipped[1] (kg)	MT Medicated Feed Disposal[2] (kg)	MT Medicated Feed On-hand (kg)	Inventoried by (initials)

[1] Unused MT medicated feed that is shipped to another facility participating in MT INAD #11-236 (Note: MT medicated feed can only be shipped to another facility with prior authorization by the AADAP Office).
[2] Unused MT medicated feed that is disposed of by incineration.

Date Prepared: _____ **Investigator:** _____

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

OMB Control No. 1018-####

Expires ##/##/20##

Form MT-2. Chemical Use Log for Clinical Field Trials Using MT Medicated Feed Under (Cont.) INAD #11-236

Quantity on Hand From Previous Page (lbs): _____ **Facility:** _____ **Reporting Individual:** _____

MT Medicated Feed Batch Number and Manufacture Date	Date Received	Amount Received (kg)	Dates Used	Study Number	MT Medicated Feed Used for Treatment (kg)	MT Medicated Feed Shipped[1] (kg)	MT Medicated Feed Disposal[2] (kg)	MT Medicated Feed On-hand (kg)	Inventoried by (initials)

[1] Unused MT medicated feed that is shipped to another facility participating in MT INAD #11-236 (Note: MT medicated feed can only be shipped to another facility with prior authorization by the AADAP Office).

[2] Unused MT medicated feed that is disposed of by incineration.

Date Prepared: _____ **Investigator:** _____

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

Form MT-2a. Daily Record of MT Medicated Feed Use (*for use as a supplement to Form MT-2*)

Instructions: 1. Form MT-2a should be used by the Investigator to supplement data on Form MT-2.
2. A separate Form MT-2a should be used for each treatment event.

Study Number	Treatment Day	Date	MT-Medicated Feed Used (kg)	Feed Administered by (initials)
	1			
xxxx	2			
xxxx	3			
xxxx	4			
xxxx	5			
xxxx	6			
xxxx	7			
xxxx	8			
xxxx	9			
xxxx	10			
xxxx	11			
xxxx	12			
xxxx	13			
xxxx	14			
xxxx	15			
xxxx	16			
xxxx	17			
xxxx	18			
xxxx	19			
xxxx	20			
xxxx	21			
xxxx	22			
xxxx	23			
xxxx	24			
xxxx	25			
xxxx	26			
xxxx	27			
xxxx	28			

Date Prepared: _____ **Investigator:** _____

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

OMB Control No. 1018-####

Expires ##/##/20##

STUDY NUMBER _____

Form MT-3: Results Report Form for Clinical Field Trials Using MT Medicated Feed Under INAD 11-236

INSTRUCTIONS

1. Investigator must fill out Form MT-3 no later than 30 days after completion of the study period. Attach lab reports and other pertinent study information.
2. If 17-alpha methyltestosterone medicated feed was not used under the assigned Study Number, contact the Study Director at the AADAP Office on how to close-out the study.
3. Investigator should forward a copy of Form MT-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

MT medicated feed batch number		MT medicated feed manufacture date	
Treatment dosage	**9 mg/kg bw/day**	Treatment duration	**28 days**
Fish species treated		Total number of fish treated	
Number of rearing units treated		Number of fish per treated rearing unit	
ID of all treated rearing units (e.g. Tank 5, Pond 6B)			
Number of control units		Number of fish per control unit	
Fish age (days post-hatch)		Average fish length (mm)	
Treatment date (initiated)		Treatment date (completed)	
Sample collection (yes/no)		Sample collection date	
Number of fish remaining at the completion of the treatment period (i.e. number of fish treated minus treatment period mortality)			

WATER QUALITY PARAMETERS

Mean Treatment Temperature (^{o}F)		Mean Dissolved Oxygen (mg/L)	
Mean pH		Mean Hardness - CaCO3 (mg/L)	

RESULTS: Describe in brief detail treatment results. Did treatment go as planned? Did all fish readily consume MT medicated feed? Was any unusual fish behavior or unexpected mortality associated with the treatment?? 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?

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

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

Observed withdrawal period for BATCH CULTURE: _____ **120 days** (Investigator should initial)

Observed withdrawal period for PARTIAL HARVEST/RESTOCK CULTURE: _____ **350 g fish weight** (Investigator should initial)

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

DISPOSITION OF MT MEDICATED FEED

☐ Use and disposition of all MT medicated feed followed Study Protocol guidelines and has been clearly identified on Form MT-2 (Investigator should initial)

NEGATIVE REPORT MT medicated feed was not used at this facility under this Study Number during the reporting period. (Investigator should initial for negative reports as soon as the Study Number is known to be no longer needed or valid.)

Date Prepared: _____ **Investigator:** _____

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

Form MT4a. Report on Efficacy (Sex Ratio) Determination Sample for Clinical Field Trials Under MT INAD 11-236 (for use by histopathologist)

INSTRUCTIONS

1. Upon receipt of a Form MT-4 from a participating facility, the Sponsor will initiate a new Form MT-4a (note: Form MT-4 must be completed for each 60 fish sample of tilapia fingerlings (≥5 cm total length) sent to the Sponsor for histological evaluation of gonads).
2. Form MT-4a should be appended to the corresponding Form MT-4.
3. Form MT-4a will be completely jointly by the Sponsor and the histopathologist.
    1. The Sponsor will provide a completed copy of Form MT-4a to the facility from which the sample originated.

1. **Note:** Both the certified histopatholgist and the Sponsor should sign and date Form MT-4a.

SITE AND TREATMENT INFORMATION

Facility			
Investigator			
MT medicated feed batch number		MT medicated feed manufacture date	
Treatment dosage	**9 mg/kg**	Treatment duration	**28 days**
Treatment date		Treatment date	
Date sample received		Date sample evaluated	

TREATMENT SUMMARY INFORMATION

Number of Fish Examined			
Number with Testis only		**Percentage Testis only**	
Number with Ovary only		Percentage Ovary only	
Number with Ovotestis		Percentage Ovotestis	
Date Summary Information Completed by Sponsor		**Sponsor Signature**	

RESULTS OF MICROSCOPIC EXAMINATION OF GONADAL TISSUE

To be completed by histopatholgist. Indicate gonadal tissue type by "**x**" in appropriate box; **T** = Testis; **O** = Ovary; **OT** = Ovotestis

Fish	T	O	OT	Fish	T	O	OT	Fish	T	O	OT
1				21				41			
2				22				42			
3				23				43			
4				24				44			
5				25				45			
6				26				46			
7				27				47			
8				28				48			
9				29				49			
10				30				50			
11				31				51			
12				32				52			
13				33				53			
14				34				54			
15				35				55			
16				36				56			
17				37				57			
18				38				58			
19				39				59			
20				40				60			

If any gross anatomical abnormalities were observed, please describe:

Name of Histopathologist (please print): _____

Date Prepared: _____ **Signature:** _____

(Histopathologist)

STUDY NUMBER _____

Form MT-5: Transfer Record of MT-treated Fingerling Tilapia Under MT INAD 11-236

INSTRUCTIONS

1. Form MT-5 must be completed by Investigators for all sales of MT-treated fingerling tilapia to other producers for grow-out of fish to market size. The purpose of this form is to formally establish that all purchasers of MT-treated fingerling tilapia are aware of, and agree to comply with, the FDA-mandated withdrawal period(s) that must be observed before MT-treated tilapia may be slaughtered for processing or released for possible human consumption.

2. This form should be completed and signed by both the Investigator and purchaser prior to the transfer of fish.

3. A copy of this form needs to be uploaded to the correct Form MT-3 in the online database or emailed to the Study Director within 10 days of fish transfer for inclusion in the permanent file.

INVESTIGATOR (i.e. seller) INFORMATION

Investigator			
Facility			
Number of MT-treated fingerlings transferred		Transfer date	
Treatment dates (i.e., start date - end date)		Study Number Corresponding to MT-treated fingerlings	

PURCHASER (i.e. buyer) INFORMATION

Name of purchaser	
Facility address	
Phone Number	

As described in the FDA-approved Study Protocol for MT INAD 11-236:

a.) The investigational withdrawal period for fish reared under a batch culture regimen will be 120 days. Batch culture is defined as when all fish in a group/lot enter and leave the lot at the same time (sometimes referred to as "all in/all out" culture). In batch culture a defined group of similar age/size fish are stocked simultaneously for a prescribed grow-out period, which is followed by a complete harvest of the production unit. This withdrawal period is determined based on the last day of treatment with 17-alpha methyltestosterone medicated feed.

b.) The investigational withdrawal period for fish reared under partial harvest/restock culture will be until such time that harvested fish reach an individual minimum weight of 350 g. Partial harvest/restock culture is defined as the mixing of different lots of fish during the grow-out period, and the selective harvest of fish from the production unit at various times.

The purchaser acknowledges his/her responsibility to comply with the above-stated withdrawal periods:

Purchaser: _____ **Date:** _____

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].

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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].