

**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, Division of National Fish Hatcheries

Sponsor Signature

Date Approved

Manufacturer (medicated feed):

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

Facility 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

September 30, 2010

Study Director

Mr. Jim Bowker

Study Director Signature

Date

Clinical Field Trial Location and Trial Number:

Type or Print Facility Name

Trial Number

Investigator _____

Type or Print Name

Investigator Signature

Date

STUDY PROTOCOL FOR 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. David Erdahl, 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: dave_erdahl@fws.gov

Manufacturer (medicated feed):

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

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

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

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: October 1, 2007

Proposed Completion Date: September 30, 2010

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-methylandro-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°C
Odor: no information available

b. Manufacturer, source of supply of 17-alpha methyltestosterone

1. Hawkins, Inc. Pharmaceutical Group
3000 East Hennepin Avenue
Minneapolis, MN 55413

Only the authorized feed manufacturer (i.e. Rangen Inc.) will be allowed to purchase, receive, and store 17-alpha methyltestosterone from Hawkins, Inc. 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.

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 Material Safety Data Sheet (MSDS) 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 MSDS. Safety precautions as outlined in the MSDS 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

Rangen Inc. will be the only entity authorized to purchase, receive, and store 17-alpha methyltestosterone, and will also be the sole supplier of 17-alpha methyltestosterone medicated feed to all Investigators under this INAD.

1. All Facilities Using 17-alpha methyltestosterone medicated feed:

Immediately upon receiving an order/shipment of 17-alpha methyltestosterone medicated feed, the Investigator will complete Form MT -1 *Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals*. The investigator will archive the original in the facilities INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form MT-1. The Study Monitor will then forward a copy to the Study Director at the AADAP Office. The Study Director will archive one copy, and send another copy of Form MT-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form MT-1s are received by the Study Director in a timely manner.

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) will be supplied to each Investigator. Each time 17-alpha methyltestosterone medicated feed is used it must be reported by the Investigator on Form MT-2.

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.

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.

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

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol 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).

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.

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* that details each planned,

specific treatment event. The worksheet should be filled out, signed, and sent by Fax to the Study Monitor. The Study Monitor will review the planned treatment (i.e. Worksheet), sign it, and forward (e.g. Fax) the Worksheet to the AADAP Office. The AADAP Office will review the Worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study Monitor of the assigned Study Number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. The Investigator should record the assigned Study Number on Forms MT-1, MT-2, MT2a, MT-3, MT-4, and MT-5, 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 a study number and permission to proceed.

X. TREATMENT GROUPS

- A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish, 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.
 - 1. 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.
 - 2. 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 regime, 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 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 minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with 17-alpha methyltestosterone medicated feed. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the 17-alpha methyltestosterone medicated feed treatment involved should be appropriately labeled.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or field 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.

A minimum of 60 fish will be sampled from a treatment to determine the sex ratio of a population. Fish sampled for determination of sex ratio must be a minimum of 5 cm in total length (i.e. ~60 days post-hatch). Fish should be collected randomly from the treatment lot by the Investigator, preserved in 10% formalin in a leak-proof container, and shipped to the Study Director. Each container of sample fish must be clearly identified/labeled with the name of the facility, name of the investigator, Study Number, and date treatment was initiated. Form MT-4 *Efficacy (Sex Ratio) Determination Sample* must also accompany the shipment. Samples should be sent to Dr. David Erdahl, U.S. Fish and Wildlife Service, AADAP Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; phone: 406-587-9265 x 125. Sex ratio determination will be conducted by microscopic evaluation of gonadal tissue by a certified fish histo-pathologist under contract by the sponsor.

It is important to note that efficacy sampling (i.e. sex ratio determination) is not required for all treatments (i.e. individual field trials) conducted under this INAD. Although participating facilities are encouraged to conduct efficacy sampling on a regular basis, **each participating facility will only be required to conduct efficacy sampling a minimum of once per calendar year.**

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
- Form MT-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
- Form MT-2. Chemical Use Log for Clinical Field Trials Using MT Medicated Feed Under INAD 11-236
- Form MT-2a. Daily Record of MT Medicated Feed Use
- Form MT-3. Results Report Form for Clinical Field Trials Using MT Medicated Feed Under INAD 11-236
- Form MT-4. Efficacy (Sex Ratio) Determination Sample for Clinical Field Trials Under MT INAD 11-236
- Form MT-4a. Report on Efficacy (Sex Ratio) Determination Sample for Clinical Field Trials under MT INAD 11-236
- Form MT-5. Transfer Record of MT-treated Fingerling Tilapia Under MT INAD 11-236
- Form MT-6. Year End Efficacy Report for Clinical Field Trials Using MT Medicated Feed under INAD 11-236

Copies of these forms are attached to this Study Protocol. The use of Forms MT-W, MT-1, MT-2, MT2a, MT-3, MT-4, MT-5, MT-6 are mandatory and the responsibility of the Investigator. Form MT-4a is for use by the Sponsor only.

XIV. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data should be

original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it; each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes; the original record should be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

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 regime 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. 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.

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 Section VII.A.6. Accountability (pages 6-7) for information and details.

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A Study Monitor should be assigned to each facility that is authorized to treat fish with 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 Section VII.B. Items needed for treatment, sample collection, observations, etc.; page 7).

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 Section VII.A.6. Accountability (pages 6-7) for details and Form MT-W, Form MT-1, Form MT-2, Form MT-2a, Form MT-3, Form MT-4, Form MT-4a, Form MT-5, and Form MT-6 for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study 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 an annual 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. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form MT-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Original raw data on Form MT-2 should be retained by the Investigator until completion of the calendar year, at which time copies should be sent to the Study Monitor. Original raw data on Form MT-2a and Form MT-3 should be retained by the Investigator until completion of the study, at which time copies should be sent to the Study Monitor. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, the Study Monitor should forward all data to the Study Director. As stated above, a complete set of raw data (including Form MT-4 and Form MT-5) should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

Form MT-3 Results Report Form is to be completed no later than 30 days after a course of therapy is completed. The purpose of this form and supplementary data is to document the completion and results of the treatment. In addition to the data solicited by the form, attach original source data that may have been collected to document any treatment effects.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the Bozeman National INAD Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual INAD report will be prepared and submitted to the FDA. 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 on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with Form MT-3, and ultimately be submitted to the Study Director.

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