

**STUDY PROTOCOL FOR AN AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION
FOR THE USE OF 17-ALPHA METHYLTESTOSTERONE IN
RAINBOW TROUT AND ATLANTIC SALMON
(INAD #8557)**

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

July 16, 2007

Proposed Ending Date

July 31, 2023

Study Director

Ms. Bonnie Johnson

Study Director Signature

Date

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 RAINBOW TROUT AND ATLANTIC SALMON UNDER INAD #8557

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of 17-alpha methyltestosterone when administered in feed to early life stage rainbow trout and Atlantic salmon to produce masculinized female fish that produce sperm. Clinical field trials will be conducted on early life stage rainbow trout and Atlantic salmon held at a various salmonid production facilities under a variety of environmental conditions under INAD #8557.

II. SPONSOR

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P.O. Box 706
Buhl, ID 83316

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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

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: July 16, 2007

Proposed Completion Date: July 31, 2025

V. BACKGROUND/PURPOSE

A. Background Information:

Sex control can be an effective tool in fish husbandry to improve product quality and profitability. In rainbow trout culture, for example, female fish are more desirable as a consumable product as a result of improved flesh quality and faster growth rates. Since relatively few male fish are required to fertilize females, reducing and optimizing the number of males in a population allows for a more productive brood station and better ecological use of fish-farm resources. Ultimately, in salmonid culture, the objective may be to eliminate genotypic males from the brood stock. Fertilization of females is then accomplished with masculinized females that produce sperm containing no Y chromosome. Please note that we have adopted the term "masculinized females" to describe the objective and successful outcome of the treatment. This term is used synonymously with previously used terms including "genotypic females" or "phenotypic males" wherever possible, unless the latter terms are required to describe a specific condition or process.

Methyltestosterone administration is an experimentally validated and efficacious method for sex control in salmonid fish. Its use is particularly attractive for the production of all female fish, because application of the compound occurs in early life stages of brood stock rather than in the consumed product, thereby effectively eliminating concern for exposure of the consumer to the compound. The application of the drug to eggs or fry results in masculinized female fish that produce sperm and, therefore, when crossed with true females, reliably produce only females, because the masculinized female sperm contains only X chromosomes.

Androgens were studied as a method to control sex differentiation in fish at least as early as 1957 (Ashby 1957). The effective use of methyltestosterone to induce the development of phenotypically male salmonids from genetically female fish has been experimentally investigated by several research groups and was reviewed by

Donaldson and Hunter (1982). Johnstone et al. (1978) reported dose efficacy experiments using rainbow trout (*Oncorhynchus mykiss*) = [*Salmo gaidneri*] and Atlantic salmon (*Salmo salar*). Methyltestosterone was incorporated into defatted commercial feeds at concentrations of 3 mg/kg and 30 mg/kg. The authors did not detect any adverse effects (excluding changes in sexual differentiation) associated with feeding these diets for up to the first 120 days of feeding. A number of dose concentration/duration combinations were performed in which varying proportions of male fish up to 100% of the population were produced. Administration of methyltestosterone to rainbow trout at 3 mg/kg concentration in feed resulted in 100% male fish. Microscopic examination of gonads from treated fish in a variety of groups demonstrated areas of normal male reproductive follicles, as well as non-differentiated areas of tissue and the less frequent observation of ovarian inclusions.

During studies conducted since 1993, under INAD 8557, we have concluded that the most effective target dosage may be achieved by using an even lower drug in feed concentration in the range of 1 mg/kg to 3 mg/kg. If dosage is excessive in rainbow trout, the likely result is sterilization of the treated female, rather than phenotypic masculinization. We will use these findings in our definitive range finding and dose confirmation studies discussed later in this document.

The results of Johnstone et al. (1978) on Atlantic salmon were less definitive, apparently due at least in part to difficulties in maintaining experimental populations of fish. Both doses of methyltestosterone resulted in either male or non-differentiated gonads at up to 9 months of age, the oldest fish examined. However, Simpson (1975/1976) succeeded in inducing 100% male Atlantic salmon by immersing eggs in 250 µg/mL methyltestosterone, followed by feeding the drug at a concentration in the diet of 30 mg/kg for the first 120 days of feeding.

Cravedi et al. (1989) used C¹⁴-labeled methyltestosterone to evaluate the metabolism of the drug in rainbow trout. About 67% of the drug was excreted as free metabolites after 24 hours. Three days after a single dose, about 20% of the ingested radioactivity was bound to tissues.

Preliminary experiments conducted at the field trial sites under both INAD 8557 and an earlier investigational permit indicate that sex reversal can usually be accomplished with greater than 90% efficacy in rainbow trout. Clearly, the experimental basis for this application of methyltestosterone is well-established, as is the lack of adverse effects. In conjunction with the inherent safety of the treatment for the consumer and the significant advantages to production of using the method, field trials leading to the registration of the compound are now justified and required.

The primary issue preventing earlier registration of 17MT for this application is the extremely small market for the compound due to the very small amounts of drug and feed formulation required to support the entire U.S. trout industry. This is not due to the small size of the industry but rather due to the small size and number of

fish that need to be treated and the potency of the drug. For example, in 1995 we estimated that 200 million trout eggs were needed by the private U.S. trout industry each year and 3 billion eggs were used world-wide. One of the participants in this project produces 340 million eggs per year. The production of this number of eggs requires approximately 2.5 gms of 17MT per year with a market value of about US \$8.50 (eight dollars and fifty cents). Scaling this use rate for U.S. and world production, the world trout industry requires a quantity of 17MT worth about US \$75.00 (seventy five dollars), and the entire U.S. trout industry requires an annual quantity of 17MT worth about US \$5.00 (five dollars).

This protocol describes the treatment of rainbow trout fry and Atlantic salmon fry which will become brood fish and subsequently produce, for sale, eggs and progeny as smolts with methyltestosterone. Therefore, in production, the drug will never be used on fish that are produced for human consumption. The elapsed time from treatment of the fry to disposal of the brood stock carcasses by approved methods is a minimum of 24 months. Because the proposed trial facilities produce only eggs and smolts, the drug is never used in the same facility where fish are eventually grown out for human consumption. The INAD under which this protocol is performed (INAD 8557) supersedes earlier INAD 4674. The use of the compound under the previous INAD has resulted in stocks of a rainbow trout at the trial site in which the proportion of true Y chromosome-bearing males has been markedly reduced. The use of the method on Atlantic salmon at the farm is more recent, and methods to optimize the treatment are still appropriate. Therefore, the protocol and data analysis will take the history of the stocks into account.

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 rainbow trout and Atlantic salmon to produce masculinized female fish that produce sperm. 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 masculinized female fish that produce sperm.

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 rainbow trout and

Atlantic salmon 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 rainbow trout and Atlantic salmon.

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: C₂₀H₃₀O₂
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. Medisca
6090 Henri-Bourassa West
St-Laurent, QC H4R 3A6

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 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

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.

The INAD Program Management System (IPMS) is an on-line database that must be used by Investigators for ALL INAD reporting. The IPMS 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 IPMS (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the IPMS) 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". The investigator must then forward Form MT-1 to the Study Director at the AADAP Office. The Study Director will in turn forward a copy 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) must be completed and maintained by each Investigator. Each time 17-alpha methyltestosterone medicated feed is used, it must be recorded 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 pertains to each specific treatment event. The worksheet should be filled out, electronically signed, and forwarded to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), electronically sign it, 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 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 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 a study number and permission to proceed.

Note: The INAD Program Management System (IPMS), which is an on-line database that 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 8557.
- 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 and duration

1. Rainbow trout

17-alpha methyltestosterone (MT) for rainbow trout will be administered at a dosage of 10.8 mg MT/kg of fish biomass. The maximum target dosage for rainbow trout is 180 µg/kg/day from first feeding for 60 days for a total estimated dose of 10.8 mg/kg. This dose is based on feed containing 3 mg/kg of drug fed at an estimated average daily ration of 6% body weight. **No re-treatment of fish will be allowed.**

2. Atlantic Salmon

17-alpha methyltestosterone (MT) for Atlantic Salmon will be administered at a dosage of 6.3 mg MT/kg of fish biomass. The maximum target dosage in feed is 60 µg/kg/day from first feeding for 105 days for a total feed dosage of 6.3 mg/kg. This dose is based on feed containing 1 mg/kg of drug fed at an estimated average daily ration of 6% body weight. **No re-treatment of fish will be allowed.**

Table 1. Trout Product: Target Drug in Feed Concentration: 3 mg/kg.						
Ration level (% body weight fed per day)	Drug fed per day per kg of fish (mg /kg/day)		Drug fed per day per kg of fish (µg /kg/day)		Target total drug fed over treatment period (60 days for trout)	
1%	0.03	mg/kg	30	µg/kg/day	1.8	mg/kg
2%	0.06	mg/kg	60	µg/kg/day	3.6	mg/kg
3%	0.09	mg/kg	90	µg/kg/day	5.4	mg/kg
4%	0.12	mg/kg	120	µg/kg/day	7.2	mg/kg
5%	0.15	mg/kg	150	µg/kg/day	9	mg/kg
Maximum target for this study: 6%	0.18	mg/kg	180	µg/kg/day	10.8	mg/kg

Table 2. Atlantic Salmon Product: Target Drug in Feed Concentration: 1 mg/kg.						
Target ration level (% body weight fed per day)	Drug fed per day per kg of fish (mg /kg/day)		Drug fed per day per kg of fish (µg /kg/day)		Target total drug fed over treatment period (105 days for Atlantic salmon)	
1%	0.01	mg/kg	10	µg/kg/day	1.05	mg/kg
2%	0.02	mg/kg	20	µg/kg/day	2.1	mg/kg
3%	0.03	mg/kg	30	µg/kg/day	3.15	mg/kg
4%	0.04	mg/kg	40	µg/kg/day	4.2	mg/kg
5%	0.05	mg/kg	50	µg/kg/day	5.25	mg/kg
Maximum target for this study: 6%	0.06	mg/kg	60	µg/kg/day	6.3	mg/kg

C. Dosing interval and repetition

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

D. 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.

E. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to 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 objective is to document the production of female offspring by fertilizing eggs from female fish with sperm from masculinized female fish, thereby resulting in all female offspring, and to progressively reduce the proportion of Y chromosome-bearing males on the field sites. In the experimental design, a proportion of fry receive the treatment, which results in a high proportion of masculinized females. Untreated fish maintain a high proportion of females, because these fish are the result of previous treatments that have resulted from the culling of true males from the population. In both rainbow trout, but more so in Atlantic salmon, there is still a small proportion of true males in the farmed population. This protocol calls for an evaluation of treatment effect between 4 and 12 months post-fertilization, at which time 60 treated and 60 untreated fish from each lot are examined by necropsy to determine by anatomical characteristics the presence of any true males in the groups. Groups that contain true males or true females are discarded.

Groups that are retained after they are judged to be masculinized females are held until about 24 months post-spawning. Just prior to spawning, individuals from this group are examined. Fish from which sperm can be manually expressed are regarded as true Y chromosome-bearing males, because they possess a spermatid duct, and are discarded. Other fish in the group that have secondary

male characteristics but from which sperm cannot be expressed are considered to be masculinized females. These fish are sacrificed and their sperm is used to fertilize eggs from females. As a result, the females crossed with the masculinized females should produce all female offspring. For production purposes, many of the offspring are sold as eggs or fry, but selected groups of some of the crosses are retained to determine the efficacy of treatment and for replacement brood stock.

In the case of Atlantic salmon stocks at the trial sites, there is a higher proportion of Y chromosome-bearing males in the total population. This is a result of the more recent initiation of the selection program and a result of the procedure used for rainbow trout, which although effective in many cases, does not appear to have as high a success rate.

Fish from which sperm can be manually expressed are deemed to be true males and are discarded. At this point, the masculinized females are anatomically distinct from the true males, because the former lack spermatid ducts and have typical “golf ball”-shaped testes. Only masculinized females are used for crossing with the true females.

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 fish, 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 8557

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 8557

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 8557

Copies of these forms are attached to this Study Protocol. Actual reporting is accomplished on forms located on the INAD Program Management System on-line database.

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located on the INAD Program Management System on-line database.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

The minimum withdrawal time is 120 days from the last day of treatment. The investigational withdrawal period may be incorporated into grow-out periods for the

treated fish.

Investigators are authorized to market for human consumption, or release into public waters for possible human food consumption, animals treated under this investigational permit that have completed the required withdrawal time.

Fish may not be rendered or otherwise managed or moved in such a way that they could enter the human food chain prior to completing the investigational withdrawal period.

After the completion of the withdrawal period, under the specific practices of the participating farms, the fish may be potentially disposed of or released from the farm as follows:

1. Fish may be sold to a rendering plant.
2. Fish may be disposed of by incineration.
3. Fish may be ensiled at pH 3.5 and the resulting product used for fertilizer.
4. Fish may be buried in a landfill subject to applicable laws and regulations for such disposal.
5. Fish may be sold as stock for fee fishing ponds, if they are in excess of the number needed for brood stock.

Any fish culled prior to completion of the withdrawal time will be disposed of by burial or incineration and cannot be released in any way that would potentially allow them to enter the human food chain.

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 (page 8) 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. Administration 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 (page 8) for details and Form MT-W, Form MT-1, Form MT-2, Form MT-2a, and Form MT-3 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, and must complete all required data forms (see Section XIII on page 15). 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.

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 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.

Literature Cited

Ashby, KR. 1957. "The effect of steroid hormones on the brown trout (*Salmo trutta*) during the period of gonadal differentiation." *J. Embryol. Exp. Morphol.*5:225-249.

Cravedi, JP, PG Delous, and D Rao. 1989. "Disposition and elimination routes of 17 α -methyltestosterone in rainbow trout (*Salmo gairdneri*). *Can. J. Fish and Aquat. Sci.* 46:159-165.

Donaldson, EM, and GA Hunter. 1982. "Sex control in fish with particular reference to salmonids." *Can. J. Fish. Aquat. Sci.* 39:99-110.

Johnstone, R, TH Simpson, and AF Youngson. 1978. "Sex reversal in salmonid culture." *Aquaculture* 13:115-134.

Simpson, TH. 1975/76. Endocrine aspects of salmonid culture. Proceedings of the Royal Society of Edinburgh (B) 75,17: 241-252.

Appendix IV. Safety Data Sheet (SDS) for 17MT

The SDS for 17MT can be found at the drug sponsors website:

https://www.medisca.com/NDC_SPECS/MUS/0086/MSDS/0086.pdf

Form MT-W: Worksheet for Designing Individual Field Trials Under MT INAD 8557

INSTRUCTIONS

1. Investigator must fill out Form MT-W for each trial conducted under this INAD **before** actual use of MT medicated feed. The Investigator is responsible for accurate completion of Form MT-W.
2. Investigator should keep the original on file, and fax a copy to the Study Monitor for review.
3. After review, the Study Monitor will fax a copy to the AADAP Office for assignment of the Study Number.
4. The AADAP Office will review the worksheet, and then fax the assigned trial Study Number to both the Investigator and Study Monitor, at which time the trial may be initiated.
5. **Note:** Both Investigator and Study Monitor should sign and date Form MT-W.

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 (mg/kg bw/day)		Treatment duration (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)	6	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: Describe in detail the purpose of the clinical trial. Study design must be carefully focused and lend itself to rigorous evaluation. If more space is required to describe study details, title additional page(s) "Study Design" and attach them to this Worksheet.

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 Material 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

- Investigator must fill out Form MT-1 **immediately** upon receipt of 17-alpha methyltestosterone medicated feed.
- 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 FDA:*

Name of Drug	17-alpha methyltestosterone medicated feed		
INAD Number	8557	Study Number	
Proposed Use of Drug	To produce masculinized female fish that produce sperm		
Date of CVM Authorization Letter	July 16, 2007		
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	8557		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	Rainbow trout - 10.8 mg/kg body weight for 60 consecutive days Atlantic salmon – 6.3 mg/kg body weight for 105 consecutive days		
Methods(s) of Administration	Medicated feed		
Withdrawal Period	120 days		

¹ To be filled out by the NIO

Investigator: _____

Date Prepared: _____

Study Monitor: _____

Date Reviewed: _____

Sponsor: _____

Date Reviewed: _____

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: _____

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

Study Number	Treatment Day	Date	MT-Medicated Feed Used (kg)	Feed Administered by (initials)
	29			
XXXX	30			
XXXX	31			
XXXX	32			
XXXX	33			
XXXX	34			
XXXX	35			
XXXX	36			
XXXX	37			
XXXX	38			
XXXX	39			
XXXX	40			
XXXX	41			
XXXX	42			
XXXX	43			
XXXX	44			
XXXX	45			
XXXX	46			
XXXX	47			
XXXX	48			
XXXX	49			
XXXX	50			
XXXX	51			
XXXX	52			
XXXX	53			
XXXX	54			
XXXX	55			
XXXX	56			

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

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

Study Number	Treatment Day	Date	MT-Medicated Feed Used (kg)	Feed Administered by (initials)
	57			
xxxx	58			
xxxx	59			
Last day for RBT treatments	60			
xxxx	61			
xxxx	62			
xxxx	63			
xxxx	64			
xxxx	65			
xxxx	66			
xxxx	67			
xxxx	68			
xxxx	69			
xxxx	70			
xxxx	71			
xxxx	72			
xxxx	73			
xxxx	74			
xxxx	75			
xxxx	76			
xxxx	77			
xxxx	78			
xxxx	79			
xxxx	80			
xxxx	81			
xxxx	82			
xxxx	83			
xxxx	84			

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

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

Study Number	Treatment Day	Date	MT-Medicated Feed Used (kg)	Feed Administered by (initials)
	85			
XXXX	86			
XXXX	87			
XXXX	88			
XXXX	89			
XXXX	90			
XXXX	91			
XXXX	92			
XXXX	93			
XXXX	94			
XXXX	95			
XXXX	96			
XXXX	97			
XXXX	98			
XXXX	99			
XXXX	100			
XXXX	101			
XXXX	102			
XXXX	103			
XXXX	104			
XXXX	105			

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

Form MT-3: Results Report Form for Clinical Field Trials Using MT Medicated Feed Under INAD 8557

INSTRUCTIONS

- Investigator must fill out Form MT-3 no later than 10 days after completion of treatment. Attach lab reports and other pertinent study information.
- If 17-alpha methyltestosterone medicated feed was not used under the assigned Study Number, contact the Study Director at the AADAP Office to close-out the study.
- Investigator should forward a copy of Form 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 (mg/kg bw/day)		Treatment duration (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)	
Number of fish entering human food chain			

WATER QUALITY PARAMETERS

Mean Treatment Temperature (°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: _____ **120 days** (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: _____