STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR THE USE OF ESTRADIOL (17β-estradiol) IN BROOK TROUT (INAD #12-671)

Sponsor:

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

_____ _____

Sponsor Signature Date Approved

Manufacturer:

Sigma-Aldrich, Inc.
3306, 3300 S 2nd Street,
St. Louis, MO 63118

Office for Coordination of 17-β estradiol (E2) INAD:

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

Proposed Starting Date	October 6, 2016
Proposed Ending Date	October 31, 2023
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-β ESTRADIOL (E2) IN BROOK TROUT UNDER INAD #12-671

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of 17-β estradiol (E2) when administered in feed to early life stage brook trout for the feminization of early stage male brook trout. Clinical field trials will be conducted on brook trout fry held at various salmonid production facilities under a variety of environmental conditions under INAD #12-761.

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:

Sigma-Aldrich Corp.
3306, 3300 S. 2nd Street
St. Louis, MO 63118
Ph: 314-771-5765
Email: cssorders@sial.com

Study Director: Ms. Bonnie Johnson, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9905; Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9911; Email: paige_maskill@fws.gov

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses. 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 6, 2016

Proposed Completion Date: October 31, 2023

V. BACKGROUND/PURPOSE

A. Background Information:

Brook trout *Salvelinus fontinalis* introduced outside of their native range often provide marginal fisheries (Rabe 1970; Donald and Alger 1989) or negatively impact native aquatic fauna (reviewed in Dunham et al. 2002) and are thus increasingly targeted for manual removal in lakes and streams across western North America (e.g., Shepard et al. 2014). Unfortunately, complete eradication of established brook trout populations is often not achieved, and empirical studies suggest that, in most instances, manual or chemical eradication of such undesirable nonnative populations is not practical for a variety of reasons (Britton et al. 2010). The development of alternative approaches for eliminating select nonnative brook trout is needed. One such alternative is a sex-skewing approach in which the anthropogenic shifting of the population sex ratio toward males, would reduce the long-term viability of an undesired population, eventually resulting in extirpation of the population.

Perhaps the most promising of such sex-skewing methods being considered, the Trojan Y Chromosome approach (hereafter TYC) relies on the development and release of males that are genetically YY rather than the typical XY arrangement (Gutierrez and Teem 2006; Teem and Gutierrez 2010). The TYC method is so named because it involves introducing stocked YY individuals capable of incorporating “hidden” Y chromosomal material into the undesirable population. Assuming that enough YY males are released into the undesirable population over time and that they successfully survive and reproduce, the population will strongly skew towards males, theoretically eradicating the wild population once the sex ration reaches 100% males (Teem and Gutierrez 2010). Text provided by Schill et al. 2016.

The use of estradiol (E2) for use to feminize male brook trout has been tested by the Idaho Department of Fish and Game. This use is still in the developmental stages but will focus on the use of 17-β estradiol (E2) in medicated feed. Feminized male brook trout have normal XY sex chromosomes, but produce eggs (which are XY) instead of milt. When these eggs (XY) are fertilized with milt from a normal male (XY), they produce offspring which are ¼ YY. These YY (Trojan) males only produce male (XY) offspring when they spawn with a normal (XX) female. The ultimate goal of this process is to develop a brook trout eradication tool. If hatchery produced YY males are repeatedly stocked into unwanted wild populations of brook trout, ultimately the entire population would become male, and successful spawning would no longer occur.

B. Purpose of INAD:

The purpose of this INAD for 17-β estradiol (E2) is to develop clinical field trial data that will be used to determine the efficacy of 17-β estradiol (E2) when administered in feed to brook trout fry for the feminization of early life stage male brook trout. These data will be used to support a new animal drug application (NADA) for 17-β estradiol (E2).

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-β estradiol (E2) medicated feed treatment for the feminization of early life stage male brook trout.

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-β estradiol to feminize early life stage male brook trout in 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-β estradiol 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-β estradiol use in brook trout.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Established Name: estradiol

Common Name: 17-beta-estradiol

Chemical Name: beta-Estradiol 17-beta-D-glucuronide

Chemical Formula: $C_{18}H_{24}O_2$

Chemical Family: Steroid

C.A.S. Registry No.: 50-28–2

Grade: USP

Form: crystalline solid

Solubility in water: soluble 3.6 mg/L (at 27°C)

Melting point: no data

Odor: no information available

b. Manufacturer, source of supply of 17-beta-estradiol

Sigma-Aldrich Corp.
3306, 3300 S 2nd St
St. Louis, MO, 63118, USA

2. Verification of Drug Integrity/Strength:

The manufacturer (Sigma-Aldrich Corp.) will provide the analytical data necessary to establish the purity of each lot/batch of 17-beta-estradiol supplied to investigators. The batch number and date of manufacture for each batch of 17-β estradiol 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 17βE2-1) will clearly identify the batch number and date of manufacture of 17-β estradiol medicated feed shipments. If the integrity of the 17-β estradiol 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 17βE2-2). All un-usable 17-β estradiol medicated feed must be destroyed by following the disposal methods described in the SDS. The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

17-β estradiol medicated feed will be stored in an appropriate container, labeled with a sticker provided by the Eagle Fish Health lab, indicating the correct dose and treatment applied to the feed. 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-β estradiol (Appendix IV). Each person involved with the study and each person who may be present during the administration of 17-β estradiol 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-β estradiol 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-β estradiol medicated feed.

5. Investigational Labeling

A copy of the label to be attached to each container of 17-β estradiol (E2) 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-β estradiol (E2) feed.

6. Accountability

Sigma-Aldrich Corp. will be the sole supplier of 17-β estradiol 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-β estradiol (E2) medicated feed:

   Immediately upon receiving an order/shipment of 17-β estradiol (E2) medicated feed, the Investigator must complete Form 17βE2-1 “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 17βE2-1s are received by the Study Director within 10 days of drug receipt.

   All Investigators are also responsible for maintaining an accurate inventory of 17-β estradiol (E2) medicated feed on-hand. A Chemical Use Log (Form 17βE2-2) must be completed and maintained by each Investigator. Each time 17-β estradiol (E2) 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 “E2 Medicated Daily Feeding Record”.

   At the conclusion of field trials, all remaining 17-β estradiol (E2) medicated feed will be disposed of by following the disposal methods in the Safety Data Sheet (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-β estradiol (E2) medicated feed must be properly recorded and accounted for on the Chemical Use Log (Form 17βE2-2). The Study Monitor will be responsible for verifying the quantity of 17-β estradiol (E2) medicated feed remaining on hand versus the amount indicated on Form 17βE2-2. **Note:** 17-β estradiol (E2) medicated feed can be transferred to other facilities that are participating under INAD 12-671. Transfers must be shown in the Drug Inventory section of the database (formerly Form

17βE2-2).

7. Preparation Procedures – medicated feed top-coated at Eagle Fish Lab, ID

Target dose: 20 mg 17-β estradiol/ kg feed

Supplies needed:

a) 20 mL of 17β-E2 solution (1 mg 17β-E2 per ml, in ethanol)
b) Tap water
c) Pipettor/pipettes or graduated cylinder (ability to accurately measure 20 mL)
d) Scale (ability to weigh up to 1 kg of feed, accurately, to the nearest gram)
e) Atomizer
f) Cookie sheets/other pans (for mixing/drying feed)
g) Fan
h) Nitrile gloves
i) Plastic bags (for storing finished feed)

Procedure:

a) Accurately weigh 1 kg of feed and spread in an even layer on drying pan.
b) Accurately measure 20 mL of 17B-E2 solution and transfer to atomizer.
c) Add 20-30mL of tap water to atomizer and shake to mix well.
d) Charge/pressurize the atomizer, then spray part of the solution on the surface of the feed.
e) Using gloved hands, mix the feed and then redistribute it in an even layer.
f) Add more 17β-E2 solution and mix again, repeating this process until all of the 17β-E2 has been used.
g) Place the medicated feed in front of a fan and begin drying.
h) Total drying time will be approximately 4 hours. Mixing feed periodically during the drying process ensures even and rapid drying and prevents clumping of feed particles.
i) Package feed in plastic bags and store in a cool, dry place prior to use.

B. Items Needed for Treatment, Data Collection, 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-β estradiol (E2) 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-β estradiol (E2) 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.) are 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 17βE2-3. 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 17βE2-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 17βE2-2 and 17βE2-3), 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-β estradiol (E2) medicated feed conducted under this study protocol for INAD 12-671.

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-β estradiol (E2) 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-β estradiol (E2) will be administered only as a medicated feed treatment. Sigma-Aldrich Corp. will be the only source of 17-β estradiol (E2) used under this INAD.

B. Dose to be administered and duration

17-β estradiol (E2) for brook trout will be administered at a dosage of 20 mg 17-β –E2/kg at 4% body weight per day to fry for 60 days. **No re-treatment of fish will be allowed**.

C. Dosing interval and repetition

17-β estradiol (E2) medicated feed will be administered as a single treatment regimen, 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-β estradiol (E2) 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-β estradiol (E2) 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-β estradiol (E2) medicated feed.

However, if concomitant therapy is required in order to protect valuable fish stocks (i.e., threatened and endangered species not for human consumption), it should be fully documented and the efficacy data from the 17-β estradiol (E2) medicated feed treatment involved should be appropriately labeled. Contact the AADAP Office for the information that will need to be provided in the Form 17βE2-3 if concomitant therapy is conducted.

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 17βE2-3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

The objective (and claim) of treatment is to achieve high rates of feminization of treated male fish treated in the hatchery at the prescribed E2 dosage. In the experimental design, either standard XY male or YY treated male fry receive the treatment ideally resulting in a high proportion of feminized or egg-producing males. Starting at first feeding, recently hatched fry are fed the medicated feed for the prescribed duration and subsequently reared on standard feed for grow-out. Treatment effect will be evaluated at 12 months post-fertilization, at which time 60 treated and 60 untreated fish (held separately in identical sized vats) will be examined by necropsy and visual gonad inspection to determine phenotype. At this time, a fin clip will be taken for subsequent genetic evaluation and gonadal material will also be sampled and subsequently examined histologically. An overall observed phenotypic sex ratio for the treatment and control groups will be calculated and compared for both the visual gonad inspection and histology data. Following genetic tissue analysis and employment of available genetic sex markers for treated fish, the feminization rate for genetically male fish will also be calculated and reported.

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 17βE2-3.**

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. 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 17βE2. **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-β estradiol (E2) 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 17βE2-W Worksheet for Designing Individual Field Trials under 17βE2 INAD 12-671 - located in the New Study Request tab

Form 17βE2-1 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 17βE2- 2 Chemical Use Log for Clinical Field Trials Using 17βE2 Medicated Feed Under INAD 12-671– located in the Manage/View Drug Inventory tab and filled out in Form 17βE2-3 to show use

Form 17βE2-2a Daily Record of 17βE2 Medicated Feed Use – located in Form 17βE2-3

Form 17βE2-3 Results Report Form for Clinical Field Trials Using 17βE2 Medicated Feed Under INAD 12-671 – located in the Active Studies table on the home page

Form 17βE2-4a Report on Efficacy (Sex Ratio) Determination Sample for Clinical Field Trials Under 17βE2 Medicated Feed INAD 12-671 (for use by histopathologist) - located in Form 17βE2-3

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

Following sex determination, the treated fish will either be euthanized and disposed of to ensure they cannot enter the food supply or maintained and integrated into the YY Brook Trout broodstock program. Treated broodstock will be retained on station permanently or will be euthanized; they will not be made available for human consumption.

Untreated progeny of 17-β estradiol (E2) treated brook trout (YY) may be released as fry, juveniles, or mature adults. **All receiving facilities of the untreated progeny must be approved by the FDA/CVM environmental team.**

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

17-β estradiol (E2) 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-β estradiol (E2) medicated feed will be disposed of by following the disposal methods in the Safety Data Sheet. Investigators should contact Study Monitors for instructions regarding drug disposal. Disposition of all 17-β estradiol (E2) medicated feed must be properly recorded and accounted for on the Chemical Use Log (Form 17βE2-2). The Study Monitor will be responsible for verifying the quantity of 17-β estradiol (E2) medicated feed remaining on hand versus the amount indicated on Form 17βE2-2. 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 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-β estradiol (E2) 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-β estradiol (E2) 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.).

D. Administration of the drug 17-β estradiol (E2) 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-β estradiol (E2) 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 17βE2-W, Form 17βE2-1, Form 17βE2-2, Form 17βE2-2a, Form 17βE2-3, and Form 17βE2-4a.

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 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 documented on Form 17βE2-3 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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Appendix I. Sponsor Contact Information for 17βE2 INAD #12-671

Sponsor: Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9904
Fax: (406) 582-0242
Email: marilyn_j_blair@fws.gov

Sponsor Address: 4050 Bridger Canyon Road, Bozeman, MT 59715

Study Director: Ms. Bonnie Johnson
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9905
Fax: (406) 582-0242
Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9911
Fax: (406) 582-0242
Email: paige_maskill@fws.gov

1

Appendix II. Study Monitors for 17βE2 INAD #12-671

Note: This information will be provided directly to CVM

1Appendix IIIa. Facilities and Names of Investigators Participating under 17βE2 INAD #12-671

Note: This information will be provided directly to CVM

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 17βE2 INAD #12-671

The SDS for 17βE2 can be found at the following website:

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E8875&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Finterface%3DAll%26term%3Destradiol%26N%3D0%26mode%3Dmatch%2520partialmax%26focus%3Dproduct%26lang%3Den%26region%3DUS%26cm_re%3DDid%2520You%2520Mean-_-estradiol-_-estrodiol

1

Appendix V. Investigational Label for 17βE2 INAD #12-671

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 17βE2 INAD #12-671

Brook trout (*Salvelinus fontinalis*)

1

Appendix VIb. Table of Facilities and Fish Stocks Treated under 17βE2 INAD #12-671

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 17βE2: Worksheet for Designing Individual Field Trials Under 17βE2 INAD 12-671

INSTRUCTIONS

1. Investigator must fill out Form 17βE2-W for each proposed treatment under this INAD **before** actual use of 17-β estradiol (E2) medicated feed.
2. Investigator should forward a copy of 17βE2-W to the Study Monitor for review.
3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of a Study Number.

SITE INFORMATION

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

FISH CULTURE AND DRUG TREATMENT INFORMATION

Manufacturer of 17βE2- medicated feed			
17βE2- medicated feed batch number		17βE2- medicated feed manufacture date	
Treatment dosage (mg/kg feed)	20	Treatment duration (days)	60
Fish species to be treated	Brook trout	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)	**4**	Estimated total weight of fish treated (kg)	
Estimated amount of 17βE2- 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 17βE2- MEDICATED FEED (Environmental Safety Considerations):

☐ Investigator should initial here to indicate awareness that 17βE2- 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 17βE2- medicated feed have read the Safety Data Sheet for 17-β estradiol (E2) 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 17βE2-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

1. Investigator must fill out Form 17βE2-1 **immediately** upon receipt of 17-β estradiol (E2) medicated feed.
2. Investigator should forward a copy of Form 17βE2-1 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-β estradiol (E2) medicated feed		
INAD Number	12-671	**Study Number**	
Proposed Use of Drug	For the feminization of early stage male brook trout.		
Date of CVM Authorization Letter	October 6, 2016		
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	12-671		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	Brook trout – 20 mg 17βE2/kg feed at 4% body weight per day to fry for 60 days.		
Methods(s) of Administration	Medicated feed		
Withdrawal Period	Untreated progeny of 17βE2 treated brook trout may be released at any age. Treated fish will be retained on station permanently or are destroyed; they will not be made available for used for human consumption.		

[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 17βE2. Chemical Use Log for Clinical Field Trials Using 17βE2 Medicated Feed Under INAD #12-671

Instructions:
1. Initiate Form 17βE2-2 immediately upon receipt of 17-β estradiol (E2) medicated feed.
2. Each lot number of 17-β estradiol (E2) medicated feed may be used for multiple treatment regimens.

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

17βE2 Medicated Feed Batch Number and Manufacture Date	Date Received	Amount Received (kg)	Dates Used	Study Number	17βE2 Medicated Feed Used for Treatment (kg)	17βE2 Medicated Feed Shipped[1] (kg)	17βE2 Medicated Feed Disposal[2] (kg)	17βE2 Medicated Feed On-hand (kg)	Inventoried by (initials)

[1] Unused 17βE2 medicated feed that is shipped to another facility participating in 17βE2 INAD #12-671 (Note: 17βE2 medicated feed can only be shipped to another facility with prior authorization by the AADAP Office).
[2] Unused 17βE2 medicated feed that is disposed of by following the SDS disposal methods.

Investigator: _____ **Study Monitor:** _____

Signature and Date

Signature and Date

Form MT-2. Chemical Use Log for Clinical Field Trials Using 17βE2 Medicated Feed Under (Cont.) INAD #12-671

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

17βE2 Medicated Feed Batch Number and Manufacture Date	Date Received	Amount Received (kg)	Dates Used	Study Number	17βE2 Medicated Feed Used for Treatment (kg)	17βE2 Medicated Feed Shipped[1] (kg)	17βE2 Medicated Feed Disposal[2] (kg)	17βE2 Medicated Feed On-hand (kg)	Inventoried by (initials)

[1] Unused 17βE2 medicated feed that is shipped to another facility participating in 17βE2 INAD #12-671 (Note: 17βE2 medicated feed can only be shipped to another facility with prior authorization by the AADAP Office).

[2] Unused 17βE2 medicated feed that is disposed of by following the SDS disposal methods..

Investigator: _____ **Study Monitor:** _____

Signature and Date

Signature and Date

OMB Control No. 1018-####

Expires ##/##/20##

Form 17βE2-2a. Daily Record of 17βE2 Medicated Feed Use (*for use as a supplement to Form 17βE2-2*)

Instructions: 1. Form 17βE2-2a should be used by the Investigator to supplement data on Form 17βE2-2.
2. A separate Form 17βE2-2a should be used for each treatment event.

Study Number	Treatment Day	Date	17βE2-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 17βE2-2a - continued. Daily Record of 17βE2 Medicated Feed Use (*for use as a supplement to Form 17βE2-2*)

Study Number	Treatment Day	Date	17βE2-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 17βE2-2a - continued. Daily Record of 17βE2 Medicated Feed Use (*for use as a supplement to Form 17βE2-2*)

Study Number	Treatment Day	Date	17βE2-Medicated Feed Used (kg)	Feed Administered by (initials)
	57			
xxxx	58			
xxxx	59			
xxxx	60			

Date Prepared: _____ **Investigator:** _____

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

STUDY NUMBER _____

Form 17βE2-3: Results Report Form for Clinical Field Trials Using 17βE2 Medicated Feed Under INAD 12-671

INSTRUCTIONS

1. Investigator must fill out Form 17βE2-3 no later than 10 days after completion of treatment. Attach lab reports and other pertinent study information.
2. If 17-β estradiol (E2) medicated feed was not used under the assigned Study Number, contact the Study Director at the AADAP Office to close-out the study.
3. Investigator should forward a copy of Form 17βE2-3 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

17βE2 medicated feed batch number		17βE2 medicated feed manufacture date	
Treatment dosage (mg/kg feed)		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)	
Total number of treated fish at end of treatment		Number of treated fish moved to broodstock program	
Number of treated progeny released			

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 17βE2 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: _____ Treated fish will be retained on station permanently or are destroyed; they will not be made available for used for human consumption.

DISPOSITION OF 17βE2 MEDICATED FEED

☐ Use and disposition of all 17βE2 medicated feed followed Study Protocol guidelines and has been clearly identified on Form 17βE2-2 (Investigator should initial)

NEGATIVE REPORT 17βE2 medicated feed was not used at this facility under this Study Number during the reporting period. The study will be closed out in the online INAD database.

Date Prepared: _____ **Investigator:** _____

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

Form 7βE2-4a. Report on Efficacy (Sex Ratio) Determination Sample for Clinical Field Trials Under MT INAD 12-671 (for use by histopathologist)

INSTRUCTIONS

1. Upon receipt of a Form 17βE2-4 from a participating facility, the Sponsor will initiate a new Form 17βE2-4a (note: Form 17βE2-4 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 17βE2-4a should be appended to the corresponding Form 17βE2-4.
3. Form 17βE2-4a will be completely jointly by the Sponsor and the histopathologist.

4.The Sponsor will provide a completed copy of Form 17βE2-4a to the facility from which the sample originated.

5.Note: Both the certified histopatholgist and the Sponsor should sign and date Form 17βE2-4a.

SITE AND TREATMENT INFORMATION

Facility			
Investigator			
17βE2 medicated feed batch number		17βE2 medicated feed manufacture date	
Treatment dosage		Treatment duration	
Treatment date (initiated)		Treatment date (completed)	
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)

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 5 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].