

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD)
EXEMPTION FOR AQUI-S®20E (eugenol)
(INAD #11-741)**

Sponsor:

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

Sponsor Signature

Date Approved

Manufacturer:

AQUI-S New Zealand, Ltd./Merck Animal Health
35500 W. 91st Street
Desoto, KS 66018

Facility for Coordination of AQUI-S®20E INAD:

Aquatic Animal Drug Approval Partnership Program
U.S. Fish and Wildlife Service
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date

August 1, 2013

Proposed Ending Date

December 31, 2026

Study Director

Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility: _____

Investigator: _____

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STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUI-S®20E (eugenol) UNDER INAD #11-741

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of AQUI-S®20E as an anesthetic for use in a variety of fish species. Clinical field trials will be conducted on various freshwater-reared and saltwater-reared finfish; sharks; and freshwater prawn at different facilities under a variety of environmental conditions under INAD #11-741.

II. SPONSOR

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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

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

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: August 1, 2013

Proposed Completion Date: December 31, 2026

V. BACKGROUND/PURPOSE

A. Background Information:

The use of anesthetics is an important tool with broad application to fisheries management programs. Most often, anesthetics are used to reduce stress associated with the handling and/or transportation of fish. Anesthetics are widely used both in the culture of captive populations, and in field situations that involve the management of wildstock fish populations. Although a number of compounds have been used in the past, currently, the only approved anesthetics for use on fish are Finquel and Tricaine-S (active ingredient methane tricainesulfonate, NADA 042-427 and ANADA 200-226, respectively). Finquel and Tricaine-S both have a withdrawal period of 21 days. This restriction requires that potential food fish must be held for a minimum of 21 days following treatment before they can be released for legal harvest or slaughtered. While both of these products have been found to be effective anesthetics for use in fish, their required 21-day withdrawal period severely restricts approved use in many situations. In contrast, a zero-withdrawal (or immediate release) anesthetic would allow food fish to be released, stocked, or slaughtered “immediately” following treatment. In numerous fisheries management programs, and particularly those involving wildstock population assessment and evaluation, there is a critical need for such an anesthetic. AQUI-S®20E has been developed in New Zealand as an anesthetic for use on food-fish with no withdrawal period. The active ingredient in AQUI-S®20E, eugenol, is used in perfumeries, flavorings, essential oils, and in medicine as a local antiseptic and anesthetic.

B. Purpose of INAD:

The purpose of this compassionate INAD for AQUI-S®20E is to develop clinical efficacy field trial data that will be used to determine the most appropriate treatment regimen for AQUI-S®20E for use as an anesthetic in a variety of fish species. These data will be used to support a new animal drug application (NADA) for AQUI-S®20E.

The U. S. Fish and Wildlife Service (USFWS) anticipates requesting the U. S. Food and Drug Administration (FDA) to grant extensions of this INAD for additional years. The USFWS believes that data from at least 1-3 treatment seasons will be required in order to adequately assess the efficacy of AQUI-S-E as an anesthetic for use in fish, and to collect sufficient data to support a NADA.

VI. SPECIFIC OBJECTIVES

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

1. Collect scientific data necessary to establish the effectiveness of AQUI-S®20E as an anesthetic in a variety of fish species 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 AQUI-S®20E as an anesthetic so that they can maintain and manage healthy stocks of fish during the period of time necessary for collection of efficacy, safety, and residue data required for an NADA for AQUI-S®20E in fish.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Chemical Name: eugenol [2-methoxy-4-(propenyl) phenol]

C.A.S. Registry No.: 97-53-0

Molecular Formula: C₁₀H₁₂O₂

Appearance: Yellow, viscous liquid

Odor: Spicy, pungent, clove-like

Specific Gravity: 1.075 to 1.095

b. Strength and dosage form

AQUI-S®20E is 10% eugenol (active ingredient). Fish are exposed by immersion bath.

c. Manufacturer, source of supply

AQUI-S New Zealand, Ltd./ Merck Animal Health
35500 W. 91st Street
Desoto, KS 66018

Contact Person at Merck Animal Health:

Jackie Zimmerman
Phone: (208) 603-0336
email: jacqueline.zimmerman@merck.com

or

Merck Animal Health Customer Service
Phone: 1-800-521-5767
email: Customerservice@merck.com

2. Verification of Drug Integrity/Strength:

The Manufacturer (AQUI-S New Zealand, Ltd.), manufactures the active ingredient for AQUI-S®20E (GMP grade eugenol) to US Pharmacopeia specifications from clove oil purchased to an in-house specification. The inert ingredients in AQUI-S®20E are purchased to USP/NF specifications. The vendors assay these ingredients and certify they meet purchase specifications.

To achieve 10% active ingredient concentration, the manufacturer weighs the eugenol then blends it with an equal weight of inert ingredients. The manufacturer records the weight of ingredients for each batch and total weight of the mixture. The date of manufacture, as well as batch and lot numbers are also recorded. Samples of batches are analyzed by AQUI-S New Zealand, Ltd., using GC-FID to verify the integrity and strength of the final product.

AQUI-S New Zealand, Ltd. will provide the analytical data necessary to establish the purity of each lot/batch of AQUI-S®20E supplied. The lot number and date of manufacture for each batch of AQUI-S®20E will be placed on the label of each container. The form *Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals* (Form AQSE-1) will clearly identify the lot number and date of manufacture of AQUI-S®20E shipments. If the integrity of the AQUI-S®20E is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded, dated, and signed in the Drug Inventory Form (Form AQSE-2). All unusable AQUI-S®20E will be disposed of by following the Safety Data Sheet (SDS).

3. Storage Conditions

AQUI-S®20E will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. AQUI-S®20E has high stability and should be stored at room temperature in a dry location away from direct sunlight. AQUI-S®20E should be stored in a secure location such as in a locked cabinet.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Safety Data Sheet (SDS) for AQUI-S®20E (Appendix IV). Each person involved with the study and each person who may be present during the use of AQUI-S®20E shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with AQUI-S®20E. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., will be worn at all times.

5. Investigational Labeling

A copy of the label to be attached to each container of AQUI-S®20E is provided in Appendix V. Although investigational labels will be affixed to AQUI-S®20E containers by the supplier, it is the responsibility of the Investigator to ensure proper labeling of all containers of AQUI-S®20E.

6. Accountability

Merck Animal Health will be the sole supplier of AQUI-S®20E to all Investigators under this INAD.

1. All facilities using AQUI-S®20E:

Immediately upon receiving an order/shipment of AQUI-S®20E, the Investigator will complete "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 AQSE -1s are received by the Study Director within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of AQUI-S®20E on-hand. A Drug Inventory Form (Form AQSE-2) must be completed and maintained by each Investigator. Each time AQUI-S®20E is used, it must be recorded by the Investigator in the Results Report form in the "Amount Of Drug Used" table.

At the conclusion of the study, all remaining AQUI-S®20E will be disposed of by following the SDS (note: unless AQUI-S®20E is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all AQUI-S®20E must be properly recorded and accounted for on the Drug Inventory Form (Form AQSE-2). The Study Monitor will be responsible for verifying the quantity of AQUI-S®20E remaining on hand versus the amount indicated on Form AQSE-2. **Note:** AQUI-S®20E can be transferred to other facilities that are participating under INAD 11-741. Transfers must be shown on Form AQSE-2.

7. Preparation Procedures

AQUI-S®20E will be prepared according to label directions for normal use. This includes accurately measuring out (by volume or weight) the calculated amount of AQUI-S®20E needed to obtain the desired treatment dose. AQUI-S®20E is "ready-for-use" as supplied by the manufacturer, and may be added directly to treatment water. Be sure to uniformly mix the AQUI-S®20E with treatment water before actual treatment of fish.

Visit the AQUI-S website to find the AQUI-S 20E calculator to help calculate the amount of AQUI-S®20E that will be needed. Select the AQUI-S 20E calculator and then fill in the fields. Please note that due to the specific gravity of AQUI-S®20E that there is a difference between the amount of AQUI-S®20E needed in grams versus milliliters. The

calculator can be found at: <https://www.aqui-s.com/calculator>.

B. Items Needed for Treatment, Data Collection, Etc.:

Treatment and diagnostic equipment should include a balance, graduated cylinder or flask, treatment tank, recovery tank, thermometer, stop watch, and a dissolved oxygen meter.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the AQUI-S®20E 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, pond, or container used to hold fish treated in the field. 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 for the current calendar year before AQUI-S®20E can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Investigator, or Study Monitor in case emergency use-pattern needs should arise (See Section XX). However, poor planning and/or a lack of preparation will not be considered an emergency situation.

B. The characteristics of the study animals (species, number, etc.) is presented in Appendix VIb.

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of temperatures and water quality parameters. Environmental conditions will be reported on Form AQSE-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 AQSE-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 AQSE-2 and AQSE -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 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.*

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 AQUI-S[®]20E as an anesthetic. As the primary use of AQUI-S[®]20E will be to facilitate handling and reduce stress to fish when they are being handled, untreated controls would in most cases be extremely impractical, as well as detrimental to fish health. However, Investigators are encouraged to record observations with respect to the behavior and physiological state of fish prior to AQUI-S[®]20E treatment. This information will provide a “pseudo-control” as to fish condition without, or prior to, AQUI-S[®]20E treatment.

Untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator; however, 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 fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Use of control groups will ensure that results of efficacy studies provide useful information that will support a NADA.

- C. 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 AQUI-S®20E. 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

AQUI-S®20E will be administered as a static immersion bath treatment. AQUI-S®20E will be prepared according to label directions for normal use. This includes accurately measuring out (by volume or weight) the calculated amount of AQUI-S®20E needed to obtain the desired treatment dose. AQUI-S®20E is “ready-for-use” as supplied by the manufacturer, and may be added directly to treatment water. Be sure to uniformly mix the AQUI-S®20E with treatment water before actual treatment of fish.

Note: An AQUI-S 20E calculator to help calculate the amount of AQUI-S® can be found at: <https://www.aqui-s.com/calculator>.

B. Dose to be administered

AQUI-S®20E should be applied as a static immersion bath at eugenol concentrations ranging from 10 - 100 mg/L (note: AQUI-S®20E is 10% eugenol as the active ingredient). Within this range, the actual concentration applied will be at the discretion of the Investigator for the **anesthesia for surgery level** and **euthanasia level**. Dosage will likely vary with respect to species, water temperature, and level of anesthesia desired.

Note: the term **handleable** (or a handleable level of sedation) is used to describe lighter sedation that is typically used when handling fish for short periods of time (i.e., lengths and weights; spawning; floy tags; PIT tags; etc). **Anesthesia** for surgery (or surgery level of sedation) is used to describe deeper levels of sedation that are required to perform surgery on fish. Typically this level of anesthesia requires a longer exposure period.

The Efficacy Technical Section has been completed for fish that will be sedated to handleable levels. Please follow the below potential label claim dose recommendations for salmonids and non-salmonids. If there are any deviations to the below dosing

recommendations then a justification is needed in Form AQSE-3.

Treatment dose and duration recommendation for freshwater or saltwater finfish for the handleable level of sedation:

Option A: Handleable sedation for salmonids is between 25 – 40 mg/L; sedation time no longer than 5 minutes; and recovery time less than 20 minutes. If treatment dose, sedation, or recovery times are not in the recommended range then a justification must be provided in Form AQSE-3.

Option B: Handleable sedation for non-salmonids is between 40 – 100 mg/L; sedation time no longer than 5 minutes; and recovery time less than 20 minutes. If treatment dose, sedation, or recovery times are not in the recommended range then a justification must be provided in Form AQSE-3.

C. Dosing interval and repetition

AQUI-S®20E will be applied as a single treatment event, and will not require repeated treatments.

D. Duration of treatment

For this protocol we refer to the lighter sedation level as **handleable** and the deeper sedation to the surgery levels as **anesthesia**. Treatment duration will be variable, and dependent on species, water temperature, and level of anesthesia required to meet handling requirements. For example, treatment to facilitate fin-clipping would likely require lighter sedation (or a handleable level of sedation) and a relatively short treatment duration (1-5 minutes), whereas treatment to facilitate implantation of radio transmitters would likely require deeper sedation of anesthesia and a relatively long treatment duration (5-10 minutes). In all cases, duration of treatment should not exceed 15 minutes. After completion of treatment and handling, fish should immediately be placed in fresh water.

Note: see section XI. Treatment Schedules B. for treatment duration recommendations.

E. Disposition of anesthetic solution

If at all possible, discharge of anesthetic solution remaining in the treatment containers following completion of treatment should be to the ground. If ground discharge is not possible, anesthetic solution may be released/mixed with facility effluent or released directly into public surface water. In situations where minimal dilution of anesthetic solution occurs prior to release to public surface waters, a pulsed-release of anesthetic solution should be employed to minimize discharge levels.

F. Detailed procedures for drug administration

Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when working with AQUI-S®20E. The amount of AQUI-S®20E necessary for each treatment should be accurately measured out (by volume or

weight) immediately prior to treatment.

Note: An AQUI-S 20E calculator to help calculate the amount of AQUI-S® can be found at: <https://www.aqui-s.com/calculator>.

G. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. There should be no other therapy during a period extending from 2 weeks prior to treatment lasting until 2 weeks after treatment. In addition to no concomitant therapy, Investigators need to keep fish cultural procedures and environmental conditions consistent following treatment with AQUI-S®20E.

An exception to this is the FDA has decided to allow the use of certain INAD treatments to be used with AQUI-S®20E treatments **provided the longest of the withdrawal periods is observed**. The INADs must be used under the conditions of the INAD protocol. If an INAD is used please note its use in Form AQSE-3 under the description of results section. **Please consult the Study Director to find out if AQUI-S®20E can be used with another drug prior to treatments.**

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 needs to be documented. All pertinent treatment response parameters should be reported on Form AQSE-3. Treatment response parameters that should be addressed include the following:

1. Primary Response Parameters

The primary treatment response parameters in this study will be a reflection of the physiological condition of fish following treatment with AQUI-S®20E. The primary physiological conditions evaluated will include when a fish is considered to be: 1) "handleable"; 2) "anesthetized"; 3) euthanized; or 4) "recovered". In most cases, dependent upon level of anesthesia desired, a study will involve either **handleable** or **anesthetized** (surgery level of sedation), and recovered. Data should be reported in time (minutes) to reach a specific level of sedation, and time to recovery from sedation.

Handleable

A fish will be considered handleable when it loses partial or total equilibrium, has slow but regular opercular rate, can be caught easily by hand, placed on a measuring board with minimal fish movement, and easily measured for length. As a general rule, a fish will be considered handleable when it can be captured and held for several seconds without difficulty. This is similar to Stages 3 - 4 of anesthesia as described by Summerfelt and Smith (1990).

The handleable level is the most common sedation level that will be used. This level will be

used for insertion of PIT, coded, or floy tags; length and weight measurements; spawning; and other fish handling procedures that don't require surgery.

Anesthetized (surgery level anesthesia)

A fish will be considered anesthetized when it loses all reflex activity. This condition generally occurs after a fish has completely lost equilibrium. As a general rule, a fish will be considered anesthetized when it can be easily held out of water, and when lifting the operculum and touching the gill lamellae does not elicit a reflexive "cough" within 5 seconds. This is similar to Stage 5 of anesthesia as described by Summerfelt and Smith, (1990).

The anesthetized level will **only** be used for surgery level sedation for fish. For example anesthetizing the fish for insertion of a radio tag would require this level of sedation. A reason for the surgery level of anesthesia should be recorded on Form AQSE-3.

Euthanized

A fish will be considered euthanized when all opercular movements have ceased for a period of at least 2 minutes. Note: **Euthanized fish must not be sent to slaughter, made available for food, or returned to a water source as streambank enhancement.**

Recovered

An anesthetized fish will be considered recovered when it exhibits normal swimming behavior, including avoidance of obstacles. For AQUIS[®]20E treatments, the fish must recover in less than 30 minutes of exposure to fresh water to be considered "recovered".

As a result of the potential diversity of treatment conditions and unique treatment response parameters (e.g., specific level of anesthesia desired) that may be involved in these studies, Investigators are encouraged to provide detailed descriptions of all study variables, including specific definitions/descriptions of level of anesthesia and criteria used to establish anesthesia levels. Investigators may also choose to create their own forms for purposes of recording source data under this INAD. **Supplementary data forms should be attached to Form AQSE-3.**

2. Secondary Response Parameters

Secondary parameters include general observations on fish behavior and response to routine culture/management activities. Secondary parameters would include such responses as feeding activity, apparent level of stress, or other negative fish behavior (including, but not limited to, gill coughing, agitation, and/or jumping when exposed to treatment). All post-treatment mortality should be documented.

3. Adverse Drug Events

Any adverse event related 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, negative responses/behavior by the fish (e.g., gill coughing, agitation, and/or jumping when exposed to treatment) or hazards to personnel. All adverse drug events must also be

documented on Form AQSE-3. It is possible adverse drug events may occur under certain environmental conditions or with respect to specific species/strains of fish. Careful observation of all treated fish for signs of any adverse reaction to treatment is extremely important, and all observations of adverse drug events should be documented. 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 has been approved and treatments are scheduled, the Investigator at each facility covered by the AQUI-S®20E INAD will need to complete the following forms:

- Form AQSE-W. Worksheet for Designing Individual Field Trials - located in the New Study Request tab
- Form AQSE-1. Report on Receipt of Drug – located in the Manage/View Drug Inventory tab
- Form AQSE-2. Chemical Use Log for Field Trials under AQUI-S®20E under INAD #11-741 – located in the Manage/View Drug Inventory tab and filled out in Form AQSE-3 to show use
- Form AQSE-3. Results Report Form for use of AQUI-S®20E under INAD #11-741 – located in the Active Studies table on the home page

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 Study Director.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. All fish treated at hatchery facilities, or of immediate hatchery origin, must be held for at least 72 hours following treatment with AQUI-S®20E before they are stocked or allowed to enter the food chain. Fish that are treated as part of field-based fisheries management activities may be released immediately following treatment.

No withdrawal period will be required for fish that will not be catchable for 72 or more hours after release or are illegal for harvest during that 72 hour period. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products. Note: Euthanized fish must not be sent to slaughter, made available for food, or returned to a water source as streambank enhancement.

For treatments where other drugs may be used at the same time as AQUI-S®20E then the longest withdrawal period must be followed.

The Investigator must record the disposition of all treated fish on Form AQSE-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

AQUI-S®20E will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or outdated AQUI-S®20E remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Drug disposal information is available in the Safety Data Sheet (SDS) located in Appendix IV of this protocol. Disposition of all AQUI-S®20E must be properly recorded and accounted for on the Chemical Use Log (Form AQSE-2). The Study Monitor will be responsible for verifying the quantity of AQUI-S®20E remaining on hand versus the amount indicated on Form AQSE-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 AQUI-S®20E 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

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. A Study Monitor will be selected by each facility that is authorized to treat fish with AQUI-S®20E 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 AQUI-S®20E itself) are already available at each participating facility. The use of anesthetics to aid in the handling of fish is a common occurrence at most fish hatcheries and in many fisheries management programs. 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 sample collection, observations, etc..).

D. Administrator of the drug

AQUI-S®20E 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). AQUI-S®20E 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 AQSE-W, Form AQSE-1, Form AQSE-2, and Form AQSE-3.

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 the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarded to FDA for review. **The requested changes cannot occur until FDA concurrence has been received.** 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 differently 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 contacted immediately for advice. **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 AQSE-3, and ultimately be submitted to the Study Director

Please note, if there are any deviations to the below dosing recommendations then a justification is needed in Form AQSE-3. Treatment dose and duration recommendation for freshwater or saltwater finfish for the handleable stage:

Option A: Handleable stage for salmonids is between 25 – 40 mg/L; sedation time no longer than 5 minutes; and recovery time less than 20 minutes. If treatment dose, sedation, or recovery times are not in the recommended range then a deviation reason must be provided in Form AQSE-3.

Option B: Handleable stage for non-salmonids is between 40 – 100 mg/L; sedation time no longer than 5 minutes; and recovery time less than 20 minutes. If treatment dose, sedation, or recovery times are not in the recommended range then a deviation reason must be provided in Form AQSE-3.

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.

References

Summerfelt, R.C. and L.S. Smith. 1990. Anesthesia, surgery, and related techniques. In: Methods for Fish Biology. C.B. Schreck and P.B. Boyle editors. American Fisheries Society. Bethesda, Maryland. pp. 213-272

Appendix I. Sponsor Contact Information for Aqui-S® 20E INAD #11-741

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

Appendix II. Study Monitors for Aqvi-S[®] 20E INAD #11-741

Note: This information will be provided directly to CVM

Appendix IIIa. Facilities and Names of Investigators Participating under Aqui-S[®] 20E INAD #11-741

Note: This information will be provided directly to CVM and Merck Animal Health

Appendix IIIb. Sample of Knowledge Required for Position of Hatchery Manager (i.e., Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal, State, Tribal, and private agencies/facilities.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.

Appendix IV. Safety Data Sheet (SDS) for AQUI-S[®] 20E INAD #11-741

The SDS for AQUI-S 20E(eugenol) can be found at the drug sponsors website <https://www.aqui-s.com/products/aqui-s-20e>

Appendix V. Investigational Label for Aqui-S[®] 20E INAD #11-741

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."

Appendix VIa. Fish Species Treated under Aqui-S® 20E INAD #11-741

All Freshwater-reared finfish

Freshwater prawn

All saltwater-reared finfish

Sharks

Appendix VIb. Table of Facilities and Fish Stocks Treated under AQUI-S® 20E INAD #11-741

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 AQSE-W: Worksheet for Designing Individual Field Trials under
AQUI-S® 20E INAD 11-741**

INSTRUCTIONS

1. Investigator must fill out Form AQSE-W for each trial conducted under this INAD **before** actual use of AQUI-S® 20E. The Investigator is responsible that Form AQSE-W is completed accurately.
2. Investigator should forward a copy of AQSE-W to the Study Monitor for review.
3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of the Study Number.

SITE INFORMATION

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

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species			
Number of treated fish			
Average fish weight (gm)		Average fish length (in)	
Estimated total weight of fish treated (lbs)			
Intended level of anesthesia (Handleable - H ; Anesthetized - AN ; Euthanized - E)			
Intended dosage (mg/L eugenol) - <100 mg/L		Planned duration of treatment (minutes) - <15 minutes	
Estimated total amount of AQUI-S® 20E needed for proposed treatment (ml)			
Anticipated date treatment will be initiated			
AQUI-S® 20E manufacturer	AQUI-S New Zealand, Ltd.	AQUI-S® 20E lot number	

STUDY DESIGN: Provide a brief description of your planned study. The description should include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by; _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

_____ Estimated time (days, months) from last treatment day to first possible harvest for human consumption

Note: Euthanized fish must not be sent to slaughter or be otherwise available for food. If another drug is used during the Aqui-S® 20E treatment then the longest withdrawal period must be followed.

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.

WORKER SAFETY CONSIDERATIONS:

Investigator should initial here to indicate that all personnel handling the drug have read the Safety Data Sheet for AQUI-S® 20E and have been provided protective equipment, in good working condition, as described in the SDS.

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

FORM AQSE-1.Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

- Investigator must fill out Form AQSE-1 **immediately** upon receipt of AQUI-S®20E.
- Investigator should forward a copy of Form AQUI-S®20E 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.

Name of Drug	AQUI-S® 20E	INAD Number	11-741
Proposed Use of Drug	Sedation/anesthesia in a variety of fish species		
Date of CVM Authorization Letter	March 29, 2018		
Date of Drug Receipt		Amount of Drug Received	
Drug Lot Number		Trial Number	
Name of Investigator			
Address of Investigator			
Location of Trial			
Pivotal Study	Yes	Non-pivotal Study	----
Approximate Number of Treated Animals		Approximate Number of Control Animals	
Number of Animals Used Previously¹			
Study Protocol Number	11-741		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	100 mg/L eugenol for 15 minutes		
Methods(s) of Administration	Immersion		
Withdrawal Period	Hatchery use = 72 hours; Field-based use = no withdrawal (immediate release) <u>Note:</u> Euthanized fish must not be sent to slaughter or be otherwise available for food If another drug is used with AQUI-S® 20E then the longest withdrawal time is needed		

¹ To be filled out by the AADAP Office

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

Date Reviewed: _____

Sponsor: _____

Form AQSE-3: Results Report Form for AQUI-S® 20E Use under INAD 11-741**INSTRUCTIONS**

1. Investigator must fill out Form AQSE-3 no later than 10 days after completion of the trial. Attach lab reports and other information.
2. If AQUI-S® 20E was not used under the assigned Study Number, contact the Study Director at the AADAP Office on how to close-out the study.
3. Investigator should forward a copy of Form AQSE-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

AQUI-S® 20E lot number		Total amount of AQUI-S® 20E used in treatments (ml)	
Fish species treated		Dosage used (mg/L eugenol)	
Average fish weight (gm)		Average fish length (in)	
Total number of treated fish		Approximate fish age (fingerling/ juvenile/adult)	
Treatment bath vol. (gal)		Number of fish/bath	
Treatment duration (minutes)		Treatment date(s)	

WATER QUALITY PARAMETERS

Ave treatment temp (°F)		Dissolved Oxygen (mg/L)	
pH		Hardness - CaCO ₃ (mg/L)	

Anesthesia Record - Version 1

INSTRUCTIONS

1. Investigator should fill out the Anesthesia Record as completely as possible.
2. Enter the number of fish in the treatment tank at one time. Dependent upon the size of the tank, fish size, etc., this number could vary from 1 to 20 or possibly even higher.
3. Enter the level of anesthesia desired. Use “H” for handleable, “AN” for anesthetized, and “E” for euthanized as described in the Study Protocol. If other measurements or parameters are used to determine level of desired anesthesia, describe anesthesia level in detail on a separate sheet of paper and attach to Form AQSE-3.
4. Use additional copies of this form if more than 20 individual treatments are involved in the trial.
5. If a different dose; level of anesthesia; or purpose (i.e., spawning, tagging, fish health) is needed then a different study number is needed.

Date	Treatment Number	Species	Fish per Treatment	Level of Anesthesia (H; AN; or E)	Aqui-S® 20E Dose (mg/L)	Time to Anesthesia (min)	Time to Recovery (min)	Observer Initials
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							

RESULTS: Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol? **Attach any supplemental reports.**

TOXICITY OBSERVATIONS: Report any apparent drug toxicity including a description of unusual fish behavior.

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

Observed withdrawal period: _____ **72 hours**
_____ **No withdrawal (immediate release; field use only)**
_____ **If another drug was used during the Aqui-S 20E study then the longest withdrawal period needs to be followed.**

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

Note: Euthanized fish must not be sent to slaughter or be otherwise available for food.

NEGATIVE REPORT AQUI-S® 20E 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:** _____