STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR REWARD® (DIQUAT DIBROMIDE) under INAD #10-969

Sponsor:

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

_____ _____
Sponsor Signature Date Approved

Manufacturer:

Syngenta Crop Protection, LLC.
P.O. Box 18300
Greensboro, NC 27419-8300

Office for Coordination of REWARD® INAD:

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

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

_____ _____

Clinical Field Trial Location:

Facility: _____

Investigator: _____

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STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR REWARD® UNDER INAD #10-969

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of REWARD® to control mortality caused by bacterial gill disease and external flavobacteriosis in a variety of finfish species. Clinical field trials will be conducted on various freshwater-reared finfish at different facilities under a variety of environmental conditions under INAD #10-969.

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

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P.O. Box 18300
Greensboro, NC 27419-8300

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c/o Steve Cosky, Product Biology Lead, Syngenta
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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

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 facilities that need to conduct “Repetitive Treatments” need to be approved by the FDA/CVM Environmental Team

prior to conducting this treatment regimen. 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. **Note:** recently FDA/CVM is requiring all new facilities to be approved prior to joining the REWARD® INAD for any use pattern. This may be temporary so contact AADAP for more information.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: August 1, 2007

Proposed Completion Date: December 31, 2026

V. BACKGROUND/PURPOSE

A. Bacterial gill disease (BGD):

Bacterial gill disease (BGD) is one of the most serious diseases of intensively cultured fish, particularly young salmonids. If BGD is not diagnosed and treated early, significant mortality may occur within a 24-h period. Affected fish stop feeding, orient themselves into the current, and swim lethargically near the surface. Microscopically, gill epithelium is hyperplastic and covered with masses of long, thin, gram-negative bacteria. Mortality is the result of damage caused by massive bacterial infection of the gills. Stressors associated with intensive culture such as crowding and low concentrations of dissolved oxygen, not only predispose fish to infection, but also accelerate mortality. Neither the stressors involved nor their modes of action are fully understood. Although no single pathogen appears to be responsible for BGD, all known agents are gram-negative bacteria including flexibacteria, flavobacteria, aeromonads, and pseudomonads (Snieszko 1981; Post 1987). The condition is complicated by the fact that inflamed gills associated with BGD are susceptible to secondary infections by opportunistic fungi (Warren 1981). Bacterial gill disease can seriously impact the survival of intensively cultured fish, including several fish species listed as threatened or endangered under the Endangered Species Act.

B. Flavobacteriosis:

Flavobacteriosis is a collective name for columnaris disease, 'saddleback' disease, bacterial cold water disease, tail rot, peduncle disease, and related infections caused by the disease organisms *Flavobacterium columnaris (Flavobacterium columnaris)*, *Flavobacterium psychrophilus* in freshwater and *Flavobacterium maritimus* in marine fish (Holt et al. 1975, 1989, 1993; Kent et al. 1989; Morrison et al. 1981; Wakabayashi et al. 1984). Initial infections of the causative agents generally begin on body surfaces.

Columnaris disease, caused by (*Flavobacterium columnaris*), is an acute to chronic bacterial infection that has been reported to cause significant mortality in a wide variety of fish species including salmonids, catfish, walleye, bait minnows, goldfish, basses, and sunfish (Post 1987). Columnaris disease can be particularly devastating in cool and warm water species. Although the optimum temperature for the occurrence of columnaris disease is approximately 28 - 30°C, epizootics often occur in cultured fishes at 10 - 17°C. Although columnaris disease seldoms occurs in waters below 10°C, highly

virulent strains of flavobacters can cause outbreaks in cooler waters. As a result, columnaris disease outbreaks are generally highest during the summer months. Stressors such as crowding and handling often predispose fish to the disease. The transmission of *F. columnaris* from fish to fish occurs directly through the water. Fish infected with the organism can harbor it over winter, with subsequent disease outbreaks occurring during the following summer (Nelson et al. 1988).

Columnaris disease typically first invades the skin of the head region, including the mouth, lips, cheeks, and gills. It also invades injuries or open wounds on the body of the fish. The type of lesions vary with the species of fish. In scaleless fish such as channel catfish, the lesions are small and circular with gray-blue necrotic centers and red margins surrounded by a ring of inflamed tissue. In scaled fish, necrotic lesions begin at the outer margin of the fins and spread toward the body. The gills may be involved and demonstrate light-colored areas at the tips of the gill filaments. As the disease progresses, gill filaments are lost to advancing necrosis and sloughing of gill tissue (Bullock et al. 1986). The bacterium may invade the blood stream through a gill or skin lesion and become systemic. Columnaris disease is usually terminal within a relatively short time following bacteremia (Post 1987).

Bacterial cold water disease (*Flavobacterium psychrophilum*) infections are similar to those of columnaris disease, but there seems to be a greater tendency for infections to fulminate to deep-seated systemic infections involving spinal cord abnormalities, erratic swimming, tail rot, and peduncle disease. The disease has been associated with deaths of under-yearling coho salmon, rainbow trout and steelhead in the Pacific Northwest (Kent et al. 1989).

C. Control of BGD and Flavobacteriosis:

Chloramine-T has been widely used and has been found to be very effective in controlling BGD and external flavobacteriosis in cultured fishes (From 1980; Bullock et al. 1991). Chloramine-t has been approved by FDA for specific indications. Please see the approved chloramine-T label to see if the fish disease is listed for an approved use. Please note that the approved drugs should be used first before an unapproved drug is tried. Reasons to use the unapproved drug would be if there were discharge restrictions for the approved drug or the approved drug may not be the “drug of choice” for all species/environmental conditions.

Anecdotal observations by hatchery managers throughout the United States indicate that Diquat treatment is also an effective method of controlling BGD and flavobacteriosis in a variety of fish species. Use through the current diquat INAD has shown that treatments between 15 – 18 mg/L for 1 – 3 hrs up to 3 consecutive or alternating days is an effective treatment dose range.

D. Purpose of INAD:

The purpose of this INAD is to develop clinical field trial data that will demonstrate the efficacy and safety of REWARD® to control mortality caused by BGD and external flavobacteriosis in a variety of cultured finfish species under a variety of environmental conditions, and at a wide range of temperatures. These data will be used to support a new animal drug application (NADA) for REWARD®. Because there are many factors that can affect the success or failure of REWARD® immersion therapy, data is needed

that will determine the best ways to use the drug. Drug dosages, treatment schedules, fish handling methods and other variables will be tested. Complete documentation of studies that are well conceived and well carried out will be of great value.

The U.S. Fish and Wildlife Service (USFWS) anticipates that it may require several years to carry out all clinical field trials and laboratory studies required to complete a New Animal Drug Application (NADA) for REWARD® to cover major aquaculture needs. Therefore, the USFWS may request that the U. S. Food and Drug Administration (FDA) grant re-authorization of this REWARD® INAD sometime in the future. In the interim, the USFWS will continue to work closely with the sponsor and other research and conservation agencies to develop other required research data to support a NADA(s) for REWARD®. Therefore, clinical field trials planned under this particular INAD are but one part of a larger coordinated and diligent inter-agency effort that will eventually meet all REWARD® NADA data requirements.

VI. SPECIFIC OBJECTIVES

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

1. Collect scientific data necessary to support pivotal efficacy trials and establish the effectiveness of REWARD® to control mortality caused by bacterial gill disease and external flavobacteriosis in a variety of freshwater-reared finfish 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 REWARD® to control mortality caused by bacterial gill disease and external flavobacteriosis so that they can maintain and manage healthy stocks of fish during the period of time necessary for collection of efficacy and safety data needed to support a NADA for REWARD® use in a variety of freshwater-reared finfish species.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Trade Name: REWARD®

Chemical Name: Diquat dibromide [6,7-dihydrodipyrido (1,2-a:2^1,1^1-c) pyrazinediium dibromide]

C.A.S. Registry No.: 85-00–7

Molecular Formula: $C_{12}H_{12}N_2Br_2$

Form: liquid (concentrate)

Color: dark red-brown

Odor: odorless

EPA Reg. No.: 10182-404

b. Strength and dosage form

REWARD® (37.3% diquat dibromide; 62.7% inerts); contains 2 pounds diquat cation per gallon as 3.73 pounds salt per gallon

c. Manufacturer, source of supply

Syngenta Crop Protection, LLC
P.O. Box 18300
Greensboro, NC 27419-8300

Note: Diquat is often available from your local farm & ranch store, veterinary supply outlet, etc. The REWARD® brand must be used under this INAD.

Contact information for Diquat at Syngenta Crop Protection, LLC:

c/o Steve Cosky, Product Biology Lead, Syngenta
Ph. 1-336-632-6000
Email: steve.cosky@syngenta.com

2. Verification of Drug Integrity/Strength:

The manufacturer (Syngenta Crop Protection, LLC) will provide the analytical data necessary to establish the purity of each lot/batch of REWARD® supplied. The lot number and date of manufacture for each batch of REWARD® 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 DQT-1) will clearly identify the lot number and date of manufacture of REWARD® shipments. If the integrity of the REWARD® is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded and dated in the Chemical Use Log (Form DQT-2). All unusable REWARD® will be disposed of by following the Material Safety Data Sheet (MSDS).

3. Storage Conditions

REWARD® will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. Containers should be stored in a cool, dry, well ventilated area away from flammable materials and sources of heat or flame. Exercise due caution to prevent damage to or leakage from the container. REWARD® 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 MSDS for REWARD® (Appendix IV). Each person involved with the study and each person who may be present during the use of REWARD® shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with REWARD®. Eye and skin contact should be avoided at all times. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., will be worn at all times. No special respiratory protection is required during normal application. However, if the concentrate is spilled and allowed to stand, it can dry to a highly irritating dust. If needed, use MSHA-NIOSH approved respirator for pesticides for spill cleanup.

5. Investigational Labeling

A copy of the label to be attached to each container of REWARD® is provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of REWARD®.

6. Accountability

Syngenta Crop Protection, LLC will be the sole supplier of REWARD® 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 REWARD®:

Immediately upon receiving an order/shipment of REWARD®, the Investigator must complete Form DQT -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 DQT-1s are received by the Study Director within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of REWARD® on-hand. A Chemical Use Log (Form DQT-2) must be completed and maintained by each Investigator. Each time REWARD® 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 field trials, all remaining REWARD® will be destroyed by following the MSDS (note: unless diquat is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer).

Disposition of all REWARD® must be properly recorded and accounted for on the Chemical Use Log (Form DQT-2). The Study Monitor will be responsible for verifying the quantity of REWARD® remaining on hand versus the amount indicated on Form DQT-2. **Note:** REWARD® can be transferred to other facilities that are participating under INAD 10-969 or transferred to approved agriculture labeled use. Transfers must be shown on Form DQT-2.

7. Preparation Procedures

REWARD® will be prepared according to label directions for normal use. This includes accurately measuring out the calculated amount of REWARD® to obtain the target dose, adding freshwater to establish at least a 10-fold dilution, thoroughly mixing the stock solution to obtain a uniform solution, and then adding and mixing the stock solution in the treatment tank water. Investigators should note that REWARD® is 37.3% diquat dibromide.

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

Treatment and diagnostic equipment should include a balance, graduated cylinder, 5 gal plastic bucket, stirring utensil, treatment tank, recovery tank, thermometer, stop watch, a dissolved oxygen meter, a compound microscope, and microscope slides.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the REWARD® 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

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol for the current calendar year before REWARD® can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, by an Investigator, or by a 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.

It is important to note that facilities that need to conduct “Repetitive Treatments” need to be approved by the FDA/CVM Environmental Team prior to conducting this treatment regimen. 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. **Note:** recently FDA/CVM is requiring all new facilities to be approved prior to joining the REWARD® INAD for any use pattern. This may be temporary so contact AADAP for more information.

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 DQT-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 DQT-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 DQT-2 and DQT-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.

E. Diagnosis of disease:

1. Diagnosis of BGD:

a. The initial indication of an outbreak of BGD involves observation of fish behavior and gross appearance. Once BGD is suspected, the investigator will confirm the presence of the disease by observing a gill squash from an

infected fish under a microscope. In most cases, diagnosis of the disease will be provided by the Investigator. If there is any question concerning the diagnosis, the Study Monitor will confirm the diagnosis. Symptoms of fish behavior and appearance suggesting the presence of BGD include the following:

(1) Fish remain near the surface and congregate near the edge of the pond or raceway.

(2) Fish piping for air (they may break the surface to obtain a mouthful of air).

(3) Fish swim slowly and aimlessly.

(4) Gills appear swollen.

(5) Opercula may not close properly (gill tissue may protrude from under the opercula).

(6) The posterior part of the head may appear thickened because of the swollen gills and protruding opercula.

(7) Gills may contain white to gray spots.

b. Further diagnosis should include observation of bacterial masses (filamentous bacteria) in a direct gill squash. Preparation of a gill squash involves removing several gill filaments with scissors and placing them on a clean microscope slide. Another clean slide is used to "squash" and macerate the gill tissue; larger material is scraped off until a thin preparation has been made. The preparation is allowed to dry completely. One of two methods can be used to fix the slide: (1) slide is passed through a flame a few times to heat fix; or (2) 3-6 drops of 90% methyl alcohol are added to the slide, a match is used to ignite it and it is allowed to cool. The slide is then stained with methylene blue (60 seconds), rinsed thoroughly with water, blotted gently, air dried, and examined under oil immersion. Observation of long filaments in gill squashes are adequate for routine diagnosis of BGD.

2. Diagnosis of Flavobacteriosis:

a. Early signs of flavobacteriosis is a thickening of mucous at various spots on the head, opercula, and fins or around skin injuries. Fish go "off feed" and fins may develop necrotic lesions on the outer edges.

b. The presumptive diagnosis of flavobacteriosis is based on the presence of long, thin, gram negative rods taken from necrotic lesions on the surface of the skin, or by squash preparations made from the affected areas on the gills. Examination of gill arches will reveal high numbers of bacterial cells massed on the tissue surface as detected in a wet mount preparation under a microscope.

c. Definitive diagnosis of flavobacteriosis requires isolation of the bacterium on *Cytophaga* or a similar medium. Identification of the colonies isolated on the media is accomplished by standard biochemical characteristics for each species.

F. Level of disease:

Ideally, there should be increased morbidity or mortality rates among fish with disease signs typical of bacterial infections that **cannot be treated by other means**. Typical disease signs should be detectable in at least a few fish and the causative bacterial agent identified. However, the level of BGD or flavobacteriosis must be low or in the early stages of development to obtain control. If the level of the disease is far advanced, complete mortality may result. Therefore, prompt diagnosis and treatment is imperative. In general, the investigator will respond with diagnosis and treatment when daily mortality rates exceed approximately 0.5% of the total fish within a treatment group. Unlike BGD which is strictly an external condition, flavobacteriosis is initially an external condition, but if not treated may become systemic and cause blood septicemia.

G. Prophylactic Treatment:

Prophylactic (preventative) treatment of fish with REWARD® **will not be allowed** under this INAD exemption.

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 REWARD® due to the following conditions:

1. Outbreaks of BGD or flavobacteriosis often occur in only one tank or raceway at a time.

2. BGD is often so virulent that epizootic-type mortality can be expected in untreated controls. Flavobacteriosis that occurs under stressful culture conditions can also result in epizootics if the disease organism is not controlled.

3. Separating diseased fish into control and treatment groups may not only increase the stress placed on fish, but may also change environmental conditions such as population density, water quality, etc. These factors may impact the rate of progression of BGD and flavobacteriosis. Although it may be possible to minimize such bias by transferring two sub-groups of "sick" fish into two separate, but equal tanks (where one group will receive treatment and the second will serve as a non-treated control), such "study design" is not an option at many facilities. Furthermore, as diseased fish are reservoirs of flavobacterial

infection, whenever fish are transferred to new rearing units, the potential for infection is increased.

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 REWARD®. 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

REWARD® may be administered as either an immersion static bath treatment or as an immersion flow-through treatment. For immersion static bath treatment, REWARD® should be pre-mixed in a small volume of water (1-5 gal) and applied to the rearing unit at a specific concentration for 1-3 hours. Upon completion of treatment, REWARD® should be rapidly flushed from the rearing unit. For flow-through treatment, a pre-mixed REWARD® solution should be metered into the incoming water supply for 1-3 hours at a flow rate adequate to achieve the desired treatment concentration for the entire treatment period. Upon completion of flow-through treatment, REWARD® should be rapidly flushed from the rearing unit.

B. Dose to be administered

1. Treatment of disease

REWARD® may be applied as an immersion static bath or immersion flow-through treatment at concentrations ranging from 15 – 18 mg/l or 28 mg/L. Within this range, the concentration applied will be at the discretion of the Investigator. However, REWARD® use will be broken down into 2 use patterns with respect to treatment dosage that include: Option A (15 - 18 mg/L); and Option B (28 mg/L). Specific restrictions regarding these treatment options are provided below.

2. Repetitive Treatment use

Repetitive Treatments (Option C) are when fish must receive multiple treatments

within a short period of time with few non treatment days between treatments. This is usually needed for facilities that are feed training their fish or have valuable fish stocks exposed to poor water conditions. Facilities that need to use “Repetitive Treatments” need to be approved by the FDA/CVM Environmental Team prior to conducting this treatment pattern. If any of the approved conditions (i.e., increased dose; increase in number of treated units; discharge of treated water; etc…) changes at your facility then a new approval will be required before new treatments can begin. Facilities will request the dosing level and duration needed in the submission sent to the FDA/CVM Environmental Team by AADAP. Once the response letter has been received back by the FDA/CVM Environmental Team, facilities will then be able to treat at the approved dosing level for their facility for Repetitive Treatment use.

Note: until your facility receives approval to conduct Repetitive Treatments then there needs to be at least 14 days between different treatment periods.

3. Prophylactic treatment

Prophylactic (or preventative) treatment **is not allowed** under this INAD.

C. Dosing interval and repetition

1. Treatment of disease

Option A (REWARD® treatment at 15 - 18 mg/L)

The recommended dosing interval will involve one treatment or repeating the initial treatment 2 or 3 times. If more than one treatment (day 1) is selected, additional treatments may be administered on consecutive or alternate days (i.e., treatment regimen #1 = day 1, day 2, and day 3; treatment regimen #2 = day 1, day 3, and day 5). Note: treatment days must either be consecutive or alternate for the number of days allowed (i.e., treatment on day 1; day 2; and day 7 is not allowed). There will be no deviations to this unless Option C is approved and followed.

Option B (REWARD® treatment at 28 mg/L)

The recommended dosing interval will involve one treatment or repeating the initial treatment 2 or 3 times. If more than one treatment is selected, additional treatments will be administered on consecutive days.

Option C (REWARD® for Repetitive Treatment use)

The repetitive dosing interval will only be allowed if your facility has been approved to conduct this treatment pattern by the FDA/CVM Environmental Team. If you receive approval for this use pattern then you will be able to conduct back to back treatments as often as your categorical exclusion letter allows you to.

D. Duration of treatment

1. Treatment of disease

Option A (REWARD® treatment at 15-18 mg/L)

REWARD® treatments at 15-18 mg/L will be 1-3 hr in duration. After completion of treatment, treatment solution should be flushed from the rearing unit.

Option B (REWARD® treatment at 28 mg/L)

REWARD® treatments at 28 mg/L will be 1 hr in duration. After completion of treatment, treatment solution should be flushed from the rearing unit.

Option C (REWARD® treatment set by FDA response letter)

REWARD® treatments will be at the dose and duration decided by the FDA Environmental Review Team in their response letter for the requesting facility.

E. 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 REWARD®. The chemical should be accurately measured for each treatment immediately prior to treatment. To aid in the uniform distribution of chemical, REWARD® should be pre-mixed in fresh water (1-5 gal) to make a REWARD® stock solution prior to addition to rearing units. Investigators should note that REWARD® is 37.3% diquat dibromide.

F. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with REWARD®.

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 REWARD® treatment involved should be appropriately labeled. Contact the AADAP Office for the information that will need to be provided in the Form DQT-3 if concomitant therapy is conducted.

An exception to this concomitant therapy is that 17-alpha methyltestosterone medicated feed treatments are acceptable to use when diquat treatments are in progress for tilpia **provided the longest of the withdrawal periods is observed**. 17-alpha methyltestosterone medicated feed must be used under the conditions of the INAD protocol. If 17-alpha methyltestosterone medicated feed is used please note its

use in Form DQT-3 under the description of results section.

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

1. Primary Parameters

Morbidity and mortality data, coupled with case history and gill squash evaluation, usually indicate when REWARD® treatment is needed. If treatment is for an identified disease condition, **source data must be collected for at least 5 days before treatment, during treatment, and for at least 10 days after the last treatment**. If treatment is initiated for the mitigation of a suspected disease condition (i.e., metaphylactic treatment), **source data should indicate fish health status prior to treatment, as well as morbidity/mortality during treatment and for 10 days following treatment**. Collection of this data is critically important in all cases. Post-mortem examinations should be performed periodically on a representative sample of fish to establish that the cause of death is in fact from the bacterial infections under study.

As a result of the potential diversity of treatment circumstances involved in these studies, Investigators are encouraged to provide copies of their own daily mortality record forms for individual rearing units. 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 DQT-3.**

2. Secondary Parameters

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

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 the fish, or hazards to the applicator. Although REWARD® has been used fairly extensively with beneficial effect, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe of all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions. **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 REWARD® INAD will need to complete the following forms:

Form DQT-W. Worksheet for Designing Individual Field Trials under REWARD® INAD 10-969 - located in the New Study Request tab

Form DQT-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 DQT-2. Chemical Use Log for Clinical Field Trials under REWARD® INAD #10-969 – located in the Manage/View Drug Inventory tab and filled out in Form DQT-3 to show use

Form DQT-3. Results Report Form for Use of REWARD® under INAD #10-969 – 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 AADAP.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. The investigational withdrawal time for channel catfish, muskellunge, tiger muskellunge, and northern pike is 5 days. All other species treated with REWARD® must be held for at least 30 days following treatment before they are stocked or allowed to enter the food chain.

No holding time is assigned for fish that have a grow-out period that exceeds 30 days. The investigational withdrawal periods may be incorporated into the grow-out period. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

If 17-alpha methyltestosterone medicated feed treatments are used when diquat treatments are in progress for tilapia then **the longest of the withdrawal periods is observed**.

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

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

REWARD® will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or outdated REWARD® 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 REWARD® must be properly recorded and accounted for on the Chemical Use Log (Form DQT-2). The Study Monitor will be responsible for verifying the quantity of REWARD® remaining on hand versus the amount indicated on Form DQT-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 REWARD® is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). The investigational drug may not be redistributed to others not specified in the Study Protocol; however, REWARD® can be transferred to approved agriculture labeled use. Transfers must be shown on Form DQT-2.

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, 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 REWARD® 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 REWARD® 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. Administrator of the drug

REWARD® 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). REWARD® 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 DQT-W, Form DQT-1, Form DQT-2, and Form DQT-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 a

field trial 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 forwarder to the 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.** Deviations should be documented on Form DQT-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.

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Appendix I. Sponsor Contact Information for Reward® INAD #10-969

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 Reward® INAD #10-969

Note: This information will be provided directly to CVM

Appendix IIIa. Facilities and Names of Investigators Participating under Reward® INAD #10-969

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.

Appendix IV. Material Safety Data Sheet (MSDS) for Reward® INAD #10-969

The MSDS for Reward® can be found at the drug sponsor's website

http://www.syngentacropprotection.com/pdf/msds/03_250717242007.pdf

Appendix V. Investigational Label for Reward® INAD #10-969

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 Reward® INAD #10-969

Freshwater-reared finfish

Appendix VIb. Table of Facilities and Fish Stocks Treated under Reward® INAD #10-969

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** d**atabase**. Any paper forms that are submitted to AADAP will be sent back to the study participants.

Form DQT-W: Worksheet for Designing Individual Field Trials under Reward® INAD 10-969

INSTRUCTIONS

1. Investigator must fill out Form DQT-W for each trial conducted under this INAD **before** actual use of Reward®. The Investigator is responsible that Form DQT-W is completed accurately.
2. Investigator should forward a copy of DQT-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 to be treated		Disease to be treated	
Average fish weight (gm)		Average fish length (in)	
No. of fish per unit (e.g. 10,000 fish/raceway)			
Number of treated units		Number of treated fish	
Number of untreated control units		Number of control fish	
Anticipated date treatment will be initiated		Anticipated number of treatments	
Duration of drug treatment (hours)		Check type of treatment	_X_ Disease control
Check type of treatment method used		_____ Flow through	_____ Standing bath
Intended drug target dosage (mg/L)	15-18 mg/L 28 mg/L	Estimated total amount of drug needed for proposed treatment (ml)	
Drug manufacturer	**Syngenta**	Drug 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

Check applicable box(es):

☐ Study Objective A - Withdrawal period of 5 days for channel catfish, muskellunge, tiger muskellunge, and northern pike.

☐ Study Objective B - Withdrawal period of 30 days for all other species.

☐ If treated with 17MT medicated feed for tilapia the withdrawal period is the longest withdrawal period after treatments.

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

WORKER SAFETY CONSIDERATIONS:

☐ Investigator should initial here to indicate that all personnel handling drug have read Material Safety Data Sheet for Reward® and have been provided protective equipment, in good working condition, as described in the MSDS.

Date Prepared: _____ **Investigator:** _____

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

FORM DQT-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 DQT-1 **immediately** upon receipt of Reward®.
2. Investigator should forward a copy of Form DQT-1 to the Study Director at the AADAP Office.

The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted to the FDA:

Name of Drug	**Reward®**	INAD Number	**10-969**
Proposed Use of Drug	Control mortality caused by bacterial gill disease, external coldwater disease, and external flavobacteriosis in a variety of fish species		
Date of CVM Authorization Letter	10/08/2020		
Date of Drug Receipt		**Amount of Drug Received**	
Drug Lot Number		**Study Worksheet Number**	
Name of Investigator			
Address of Investigator			
Location of Trial			
Pivotal Study		Non-pivotal Study (yes/no)	
Approximate Number of Treated Animals		**Approximate Number of Control Animals**	
Number of Animals Used Previously[1]			
Study Protocol Number	10-969		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	15-18 mg/L = 3 hr 28 mg/L = 1 hr		
Methods(s) of Administration	Immersion (static bath or flow-through treatment)		
Withdrawal Period	5 days for channel catfish, muskellunge, tiger muskellunge, and northern pike; 30 days for all other fish species 17MT medicated feed used for tilapia then longest withdrawal period is needed		

[1] To be filled out by the NIO

Date Prepared: _____ **Investigator:** _____

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

Date Reviewed: _____ **Sponsor:** _____

Form DQT-2: Chemical Use Log for Clinical Trials Using Reward® INAD 10-969

INSTRUCTIONS

1. Investigator should initiate a **new** form DQT-2 **immediately** upon receipt of each shipment of Reward®.
2. Each lot number of Reward® may be used for multiple treatment regimes.

Qty of Reward® from previous page (gal) _____ Facility _____ Reporting individual_____

Date	Amount of new Reward® received (ml)	Lot number of Reward® received	Study Number	Amount Reward® used in treatment (ml)	Reward® transferred (ml)	Reward® discarded (ml)	Reward® remaining on hand (ml)	Inventory by (initials)
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						

Date Prepared: _____ **Investigator:** _____

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

STUDY NUMBER _____

Form DQT-3: Results Report Form for Use of Reward® under INAD 10-969

INSTRUCTIONS

1. Investigator must fill out Form DQT-3 no later than 30 days after completion of the study period. Attach lab reports and other pertinent information.
2. If Reward® 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 DQT-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	

TREATMENT INFORMATION AND SCHEDULE

Drug lot number		Total amount drug used (kg)	
Fish species treated		Reward[7] dosage used (mg/L)	
Treatment duration (hrs)		Number of treatments	
Disease treated		Disease diagnosed by	
Average fish weight (gm)		Average fish length (in)	
Number of fish per unit (e.g. 10,000 fish/raceway)			
Number of treated units		Total number of treated fish	
Number of control units		Total number of control fish	
Check type of treatment	_____ Flow through	_____ Standing bath	
Dates treatment started (**disease control only**)			

WATER QUALITY PARAMETERS

Ave treatment temp (°F)		Dissolved Oxygen (mg/L)	
pH		Hardness - $CaCO_3$ (mg/L	

Daily Mortality Record

INSTRUCTIONS

1. Investigator should fill out the Daily Mortality Record as completely as possible.
2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is Treated or Control, and the number of fish in each rearing unit.
3. Water temperature and individual tank mortality should be recorded on a daily basis.
4. If treatment is on 3 consecutive days, fill in only days 1-3 of the "treatment period" and proceed directly to day 1 of the "post-treatment period". If treatment is on 3 alternate days, fill in days 1-5 of the "treatment period" and proceed to day 1 of the "post-treatment period". If less than 3 treatments are used, proceed directly to day 1 of the "post-treatment period" after the final treatment. Please mark all treatment days with an asterisk.
5. **Even if mortality is zero an entry is still needed for that day.**

FACILITY										
	Rearing Unit ID									
	Treated or Control									
	Number of Fish									
	Day	Date	Water Temp (F°)	Mortality	Mortality	Mortality	Mortality	Mortality	Mortality	Daily Observer Initials
Pre-treatment Period	1									
	2									
	3									
	4									
	5									
Treatment period	1									
	2									
	3									
	4									
	5									
Post-treatment period	1									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									

STUDY NUMBER _____

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?

Pathology Report: Attach pathology report to this form. Report should include: 1) a description of how the pathogen(s) was identified; 2) disease identification records that confirm the presence of the pathogen; and 3) the name and title of the individual performing the diagnosis.

Pathology Report included: ☐ pre-treatment ☐ post-treatment

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

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

Observed withdrawal period: _____ **5 days**; channel catfish, muskellunge, tiger muskellunge, and northern pike

Observed withdrawal period: _____ **30 days**; all other fish species

Observed withdrawal period: _____ **120 days**; if 17MT medicated feed is used with tilapia

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

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

Date Prepared: _____ **Investigator:** _____

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