

**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, Division of Fish Hatcheries

\_\_\_\_\_  
Sponsor Signature

\_\_\_\_\_  
Date Approved

**Manufacturer:**

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

**Facility for Coordination of REWARD® INAD:**

USFWS's Aquatic Animal Drug Approval Partnership Program  
4050 Bridger Canyon Road  
Bozeman, Mt 59715

Proposed Starting Date	August 1, 2007
Proposed Ending Date	July 31, 2010
Study Director	Mr. Jim Bowker

\_\_\_\_\_  
Study Director Signature

\_\_\_\_\_  
Date

**Clinical Field Trial Location and Trial Number:**

\_\_\_\_\_  
Type or Print Facility Name

\_\_\_\_\_  
Trial Number

Investigator \_\_\_\_\_  
Type or Print Name

\_\_\_\_\_  
Investigator Signature

\_\_\_\_\_  
Date

# STUDY PROTOCOL FOR 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. INAD #10-969

## II. SPONSOR

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: [dave\\_erdahl@fws.gov](mailto:dave_erdahl@fws.gov)

**Manufacturer:** Syngenta Crop Protection, Inc.  
P.O. Box 18300  
Greensboro, NC 27419-8300

### Contact Person at Syngenta Crop Protection, Inc.:

Dennis Tierney  
P.O. Box 18300  
Greensboro, NC 27419-8300  
Ph. 1-800-334-9481 ext. 2850

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

**Field Trial Coordinator:** Bonnie Johnson, USFWS - AADAP

**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, 2007

Proposed Completion Date: July 31, 2010

## 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 *Flavobacter columnaris* (*Cytophaga columnaris*), *Flavobacter psychrophilus* in freshwater and *Flavobacter 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 (*Cytophaga 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 seldom 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 *C. 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 (*Flavobacter psychrophilus*) 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 chord 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). However, chloramine-t has not been approved for such use by FDA. Additionally, while chloramine-t has demonstrated a relatively broad spectrum of efficacy in a variety of fish species, it 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. General recommendations for cool and warmwater species include immersion treatment at 6-28 mg/L for 1-4 hours (personal communication, Larry Willis, ILDNR). Diquat has also been used to treat salmonids at 2 ppm for 1 hr (Warren, 1981). These anecdotal observations are supported by data that has been collected under Diquat INAD 8110, which has been held by the State of Illinois since the 1980's. Diquat use under INAD 8110 has been reported to be very effective, and to have a high margin of safety (personal communication, Larry Willis, ILDNR).

#### 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<sup>®</sup> 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<sup>®</sup>. Because there are many factors that can affect the success or failure of REWARD<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> INAD sometime in the future. In the interim, the USFWS will continue to work closely with the sponsor, the National Coordinator for Aquaculture New Animal Drug Applications, and other research and conservation

agencies to develop other required research data to support a NADA(s) for REWARD<sup>®</sup>. 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<sup>®</sup> 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<sup>®</sup> to control mortality caused by bacterial gill disease and external flavobacteriosis in a variety of finfish 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 REWARD<sup>®</sup> 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<sup>®</sup> use in a variety of finfish species.

## VII. MATERIALS

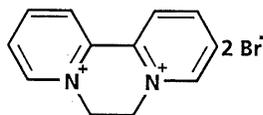
### A. Test and control articles:

#### 1. Drug Identity

##### a. Active ingredient

Trade Name:	REWARD <sup>®</sup>
Chemical Name:	Diquat dibromide [6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide]
C.A.S. Registry No.:	85-00-7
Molecular Formula:	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> Br <sub>2</sub>

Chemical Structure:



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, Inc.  
P.O. Box 18300  
Greensboro, NC 27419-8300  
Phone: 1-800-334-9481

**Contact Person for Diquat at Syngenta Crop Protection, Inc.:**

Dennis Tierney  
P.O. Box 18300  
Greensboro, NC 27419-8300  
Ph: 1-800-334-9481 ext. 2850

The shipment procedure for REWARD® is as follows: Syngenta Crop Protection, Inc. to Investigators (See Section VII.A.6 Accountability [page 7] for details and Appendix IIIa for names and addresses of Investigators).

2. Verification of drug integrity/strength:

The manufacturer (Syngenta Crop Protection, Inc.) 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, dated, and signed in the Chemical Use Log (Form DQT-2). The Study Monitor assigned to the Investigator involved will be immediately notified and the remaining material will be returned to the Study Monitor along with the properly recorded Form DQT-1.

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 Material Safety Data Sheet (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<sup>®</sup>. 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<sup>®</sup> is provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of REWARD<sup>®</sup>.

#### 6. Accountability

Syngenta Crop Protection, Inc. will be the sole supplier of REWARD<sup>®</sup> to all Investigators under this INAD.

##### 1. USFWS and Non-USFWS Facilities

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

All Investigators are also responsible for maintaining an accurate inventory of REWARD<sup>®</sup> on-hand. A Chemical Use Log (Form DQT-2) will be supplied to each Investigator. Each time REWARD<sup>®</sup> is used, it must be reported by the Investigator on Form DQT-2.

#### 7. Preparation Procedures

REWARD<sup>®</sup> will be prepared according to label directions for normal use. This includes accurately measuring out the calculated amount of REWARD<sup>®</sup> 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<sup>®</sup> is 37.3% diquat dibromide.

#### B. Items Needed for Treatment, Sample Collection, Observations, 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.

### **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 this Study Protocol before REWARD<sup>®</sup> 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).

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

### **C. 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, 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.

D. 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.

E. Prophylactic Treatment:

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

F. 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.

G. 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 Study Protocol" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent by Fax to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward (Fax) the paperwork to the AADAP Office. The AADAP Office will then review the worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. The Investigator should record the assigned study number on Form DQT-3, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, and infection/disease/treatment need is rapidly escalating, the Investigator should contact the AADAP Office for a study number and permission to proceed.

## X. TREATMENT GROUPS

- A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish, or it may be individual animals.
- B. Non-treated control groups will not be a requirement for clinical field trials evaluating the efficacy of REWARD<sup>®</sup> 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.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly

encouraged whenever circumstances permit. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals. Assignment to control and treatment groups should be random and designed to avoid bias. It is important that all 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.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which treatment response observations are recorded by individuals who are unaware which fish have been treated, at what dosage levels fish have been treated, and/or which fish are controls.

## XI. TREATMENT SCHEDULES

### A. Route of administration

REWARD<sup>®</sup> may be administered as either an immersion static bath treatment or as an immersion flow-through treatment. For immersion static bath treatment, REWARD<sup>®</sup> should be pre-mixed in a small volume of water (1-5 gal) and applied to the rearing unit at a specific concentration for 1-4 hours. Upon completion of treatment, REWARD<sup>®</sup> should be rapidly flushed from the rearing unit. For flow-through treatment, a pre-mixed REWARD<sup>®</sup> solution should be metered into the incoming water supply for 1-4 hours at a flow rate adequate to achieve the desired treatment concentration for the entire treatment period. Upon completion of flow-through treatment, REWARD<sup>®</sup> should be rapidly flushed from the rearing unit.

### B. Dose to be administered

#### 1. Treatment of disease

REWARD<sup>®</sup> may be applied as an immersion static bath or immersion flow-through treatment at concentrations ranging from 2-28 mg/L. Within this range, the concentration applied will be at the discretion of the Investigator. However, REWARD<sup>®</sup> use will be broadly broken down into 2 use patterns with respect to treatment dosage that include: Option A (2-18 mg/L); and Option B (19-28 mg/L). Specific restrictions regarding these treatment options are provided below.

#### 2. 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 2-18 mg/L)

The recommended dosing interval will involve one treatment or repeating the initial treatment 2, 3, or 4 times. If more than one treatment (day 1) is selected, additional treatments may be administered on consecutive or alternate days (e.g. treatment regime #1 = day 1, day 2, day 3, and day 4; treatment regime #2 = day 1, day 3, day 5, and day 7).

Option B (REWARD® treatment at 19-28 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 should be administered on consecutive days.

D. Duration of treatment

1. Treatment of disease

Option A (REWARD® treatment at 2-18 mg/L)

REWARD® treatments at 2-18 mg/L will be 1-4 hr in duration. After completion of treatment, treatment solution should be flushed from the rearing unit.

Option B (REWARD® treatment at 19-28 mg/L)

REWARD® treatments at 19-28 mg/L will be 30-60 min in duration. After completion of treatment, treatment solution should be flushed from the rearing unit.

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, it should be fully documented and the efficacy data from the REWARD® treatment involved should be appropriately labeled.

## XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or field management practices that indicate treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form 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<sup>®</sup> 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 at least 10 days following treatment, or until the suspected period of increased disease risk has passed.** If the period of suspected disease risk lasts for a considerable period of time, the Investigator may choose to record mortality only on days that mortality actually occurs (to save space on Form DQT-3). 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<sup>®</sup> 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 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
- Form DQT-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
- Form DQT-2. Chemical Use Log for Clinical Field Trials under REWARD® INAD #10-969
- Form DQT-3. Results Report Form for Use of REWARD® under INAD #10-969

Copies of these forms are attached to this Study Protocol.

### **XIV. RECORD KEEPING PROCEDURES**

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

### **XV. DISPOSITION OF INVESTIGATIONAL ANIMALS**

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 withdrawal period will be required for fish that will not be catchable for 30 or more days after release or are illegal for harvest during that 30 day period. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

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 out-dated REWARD® remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. The investigational drug may not be redistributed to others not specified in the Study Protocol.

## **XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES**

### **A. Drug distribution**

See Section VII.A.6. Accountability (page 7) 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. There is one Study Monitor assigned to each facility that is covered by the REWARD® 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 therapeutants to mitigate the effect of pathogens or disease 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 sample collection, observations, etc., page 7).

### **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 Section VII.A.6. Accountability (page 7) for details and Form DQT-W, Form DQT-1, Form DQT-2, and Form DQT-3 for actual forms to be used in the study.

#### F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitors who will ensure that all required information is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare an annual report that will be submitted to the FDA. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

#### G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form DQT-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Original raw data on Form DQT-2 should be retained by the Investigator until completion of the calendar year, at which time copies should be sent to the Study Monitor. Original raw data on Form DQT-3 should be retained by the Investigator until completion of the study, at which time copies should be sent to the Study Monitor. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, Study Monitors should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

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

### **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. An annual INAD report will be prepared and submitted to the FDA. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report

for submission to support a full NADA.

## **XIX. PROTOCOL AND PROTOCOL AMENDMENTS**

A signed copy of the Study Protocol must be retained by each Investigator. At any time before 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). 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 the quarterly data summaries, and ultimately be submitted to the Study Director.

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