

**STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL  
NEW ANIMAL DRUG (INAD) EXEMPTION FOR OXYTETRACYCLINE  
(TERRAMYCIN<sup>®</sup> 200 for Fish) MEDICATED FEED (INAD #8069)  
[Note: INAD 8069 Only For Use in Marine Penaeid Shrimp]**

**Sponsor:**

U.S. Fish & Wildlife Service, Fisheries and Habitat Conservation

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

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

**Manufacturer:**

Phibro Animal Health  
75 Challenger Road  
Ridgefield Park, NJ 07660

**Facility for Coordination of Terramycin<sup>®</sup> 200 for Fish INAD:**

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

Proposed Starting Date: January 1, 2011

Proposed Ending Date: December 31, 2015

Study Director: Mr. Jim Bowker

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

\_\_\_\_\_  
Date

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

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

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

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

\_\_\_\_\_  
Date

**STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR TERRAMYCIN<sup>®</sup> 200 FOR FISH MEDICATED FEED UNDER INAD #8069**

**I. STUDY ID AND TITLE:**

Clinical field trials to determine the efficacy of Terramycin<sup>®</sup> 200 for Fish when fed as a medicated feed to control mortality caused by bacterial diseases in marine penaeid shrimp species (primarily species of the genera *Litopenaeus*, *Farfantepenaeus* & *Penaeus*).

**II. SPONSOR:**

Dr. David Erdahl, U.S. Fish & 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:** Phibro Animal Health  
65 Challenger Road, 3rd Floor  
Ridgefield Park, NJ 07660.

**Contact:** Paul Duquette  
Phone: 201-329-7375  
Email: [paul.duquette@pahc.com](mailto:paul.duquette@pahc.com)

**Study Director:** Mr. Jim Bowker, U.S. Fish & 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)

**Principal Clinical 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: January 1, 2011

Proposed Completion Date: December 31, 2015

## **V. BACKGROUND/PURPOSE:**

In addition to the use patterns listed on the current label, oxytetracycline has often been the drug of choice when diagnostic evidence shows that penaeid shrimp populations (marine shrimp of the genera *Litopenaeus*, *Penaeus* and *Farfantepenaeus*) are afflicted with vibriosis, necrotizing hepatopancreatitis (NHP) or other bacterial diseases. Discounting viral diseases, the two most important maladies of shrimp are caused by bacteria of the genus *Vibrio*, and the unclassified Gram-negative, pleomorphic, intracellular Alphaproteobacterium (the etiological agent of NHP).

Integrated aquatic animal health management practices usually prevent the occurrence of these diseases. However, adverse environmental conditions, physiological changes associated with molting or spawning, uncontrollable water conditions and unforeseen factors can lead to severe disease outbreaks requiring prompt treatment to prevent significant losses. Such treatment also reduces the discharge of infectious agents into the natural environment thereby reducing the spread of disease.

Treatment strategies for the use of Oxytetracycline (Terramycin<sup>®</sup> 200 for Fish; hereafter referred to as T200) in penaeid shrimp shall be designed to meet the needs of each species or lot, the size and numbers of shrimp to be treated, the layout of the facility, and environmental conditions. In all cases, the objective shall be to minimize the impacts of disease on shrimp health, shrimp quality and survival, and to fully meet aquaculture objectives. Because there are many factors that can affect the success or failure of T200 therapy, data are needed to determine the best ways to use the drug to obtain effective disease control in route to developing an extended label claim. Complete documentation of studies that are well conceived and well carried out will be of great value.

The primary purpose of this Investigational New Animal Drug (INAD) exemption application is to obtain additional clinical field trial data to demonstrate the efficacy and target animal safety of T200 therapy to control mortality caused by bacterial diseases of marine penaeid shrimp that may occur in a variety of environmental conditions, over a range of temperatures, and in a variety of species. Specifically, the objective of clinical field efficacy trials is to evaluate the efficacy of T200 medicated feed treatment to control mortality in marine penaeid shrimp species caused by diseases susceptible to oxytetracycline. Efficacy trials will be conducted at a number of different study sites, on several species infected with a variety of bacterial shrimp pathogens. Primary diseases of interest include, but are not limited to: 1) vibriosis and 2) necrotizing hepatopancreatitis.

The U.S. Fish & Wildlife Service (USFWS) anticipates that it may require several years to carry out all clinical field trials and laboratory studies required to extend and expand the current label to cover major aquaculture needs. Therefore, the USFWS may request that the U. S. Food and Drug Administration (FDA) grant re-authorization of this T200 medicated feed INAD sometime in the future. In the interim, the USFWS will continue to work closely with the sponsor and other research entities to develop other required New Animal Drug Application (NADA) research data to support expanded labels claims for T200. Therefore, clinical field trials planned under this particular INAD are but one part of a larger nationally coordinated effort that will eventually meet all T200 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 to further establish the effectiveness of T200 to control bacterial diseases of marine penaeid shrimp species that occur under a variety of environmental conditions and over a range of temperatures.
2. Provide an opportunity for aquaculturists to legally use T200 medicated feed to control bacterial diseases of marine penaeid shrimp that occur under a variety of environmental conditions and over a range of temperatures, such that they can maintain healthy stocks of shrimp during the period of time necessary for collection of data that will be used to support expanded label claims for the use of T200 on marine penaeid shrimp.

The specific study objective of this study protocol is as follows:

Determine the efficacy of the "standard dosage and duration" of T200 therapy for the control of mortality caused by at least two of a variety of bacterial pathogens sensitive to oxytetracycline in species of marine penaeid shrimp (see Appendix VIa) reared in a range of water temperatures and under a variety of rearing or environmental conditions. An investigational withdrawal period of 2 days is required for penaeid shrimp treated in clinical field trials under this INAD until research studies demonstrate that a shorter withdrawal period meets FDA requirements for the release or slaughter of treated shrimp. No withdrawal period is required for shrimp that are to be rendered.

## VII. MATERIALS:

### A. Test and Control Articles:

#### 1. Drug Identity

##### a. Active ingredient

Common Name: Oxytetracycline ( from oxytetracycline dihydrate)

Product Name: **Terramycin® 200 for Fish** (Type A Medicated Article; i.e., premix)

Chemical Family: Tetracycline derivative

CAS Number: 79-57-2

Appearance: Uniform tan meal

Odor: Cereal odor

##### b. Strength and dosage form

T200 is a broad-spectrum anti-infective with a specially designed formula for aquatic species. It has been proven highly effective in controlling diseases caused by Gram-positive and Gram-negative organisms that adversely affect salmonids, catfish, and lobsters. **T200 contains 200g oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article.**

c. Manufacturer, source of supply

Phibro Animal Health  
65 Challenger Road  
Ridgefield Park, NJ 07660

Contact Person: Paul Duquette  
Phone: 201-329-7375  
Email: paul.duquette@pahc.com

2. Verification of drug integrity/strength:

The Manufacturer, Phibro Animal Health, will provide the analytical data necessary to establish the purity of each lot of T200 Type A Medicated Article supplied. The lot number and date of manufacture for each batch of T200 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 OTCS-1) will clearly identify the lot number and date of manufacture of T200 shipments (i.e., Type A Medicated Article or medicated feed). If the integrity of the T200 is compromised (i.e., by spilling or contamination of the stock container or feed bags) the event will be carefully recorded, dated, and signed in the Chemical Use Log (Form OTCS-2a and/or Form OTCS-2b). The Study Monitor assigned to the Investigator involved will be immediately notified.

Under INAD 8069 Investigators may either 1) purchase T200 medicated feed from a commercial feed manufacturer, or 2) prepare their own T200 medicated feed by top-coating feed on-hand (or specially ordered feed) with T200. Regardless of the source of T200 medicated feed, Investigators are encouraged (but not required) to have a sample of medicated feed assayed for oxytetracycline concentration by a certified, analytical testing laboratory. Results of T200 medicated feed assays should be appended to Form OTCS-3.

3. Storage Conditions

T200 Type A Medicated Article must be stored in a secure location in the original container supplied by the Manufacturer with the appropriate investigational label attached. The container should be stored out of direct sunlight in a well ventilated area at room temperature. The storage unit for T200 Type A Medicated Article must be labeled to indicate that it contains hazardous material and that "*NO Food or Drink is to be Stored in this unit*". T200 medicated feed should be stored in a secure location at temperatures and for periods of time not to exceed limits set by the feed manufacturer. The appropriate investigational label should be affixed to all bags/containers of T200 medicated feed. Medicated feed should be ordered only as needed and not stored for possible future use.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for T200 Type A Medicated Article (see Appendix IV). Each person involved with the study and each person who may be present during the use of T200 medicated feed shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with T200.

## 5. Investigational labeling

Copies of the labels to be attached to each container of T200 Type A Medicated Article and all bags of T200 medicated feed are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of T200 Type A Medicated Article and T200 medicated feed.

## 6. Accountability

Phibro Animal Health will be the sole supplier of T200 Type A Medicated Article to all Investigators under INAD 8069.

### 1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of T200 Type A Medicated Article or T200 medicated feed, the Investigator will complete Form OTCS-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 OTCS-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 two copies of Form OTCS-1 to FDA. Arrangements should be made between Investigators and Study Monitors to ensure completed Form OTCS-1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of T200 Type A Medicated Article and/or T200 medicated feed on-hand. Chemical Use Logs (Forms OTCS-2a and OTCS-2b) will be supplied to each Investigator. Each time T200 Type A Medicated Article and/or T200 medicated feed is used, it must be recorded by the Investigator on Form OTCS-2a and/or Form OTCS-2b, respectively.

## 7. Preparation Procedures

Oxytetracycline will be supplied to Investigators either as T200 Type A Medicated Article or T200 medicated feed. Neither product should be adulterated in any manner prior to use. If Investigators are using T200 Type A Medicated Article to make their own oxytetracycline medicated feed, T200 Type A Medicated Article should be top-coated on feed. Top-coating procedures should include "finishing" with 0.5% vegetable oil.

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

### 1. Control of bacterial disease

Sampling techniques and diagnostic equipment will most likely be provided by trained shrimp health professionals serving as Study Monitors or their designee(s). Equipment and supplies needed would include items to sample, culture, grow and identify culture growths microscopically. Standard shrimp culture supplies and equipment would also be required.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by Oxytetracycline INAD 8069 will need to complete several forms. These forms are described in Section XIII (p. 12). Copies of these forms are attached to this Study Protocol.

## VIII. EXPERIMENTAL UNIT

The experimental unit in these clinical field trials will consist of contained or isolated groups of penaeid shrimp. This will generally be groups of penaeid shrimp contained in tanks, raceways, or ponds. However, the experimental unit in clinical field trials may also be **individual animals**. If individual animals are considered to be the experimental unit, treatment response parameters for each animal must be evaluated separately.

## IX. ENTRANCE CRITERIA

### A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol before T200 medicated feed can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (See Section XX).

### B. The characteristics of the study animals (species, number, etc.) are presented in Appendix VIa and Appendix VIb.

### C. Environmental conditions

Environmental conditions will be variable and include a spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on a Form OTCS-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).

### E. Pathogen/disease considerations

1. Bacterial shrimp pathogens should be presumptively identified by procedures described in *“Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp,”* World Aquaculture Society, 2001). Other, more sensitive methods described elsewhere in peer-reviewed references, or as mutually determined by the local shrimp health professional, in consultation with the Study Monitor, also may be used. (**Note: Diagnostic methods other than those in the *“Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp”* should be described on a separate sheet attached to a Form OTCS-3 “Results Report Form”**).
2. There should be increased mortality rates among shrimp in a rearing unit(s) for three or more consecutive days. (Note: Station history and the

experience of the investigator, monitor, or the shrimp health professional may override this criterion to halt potentially explosive disease outbreaks. In such cases, however, careful diagnostic surveillance should be carried out in all rearing units proposed for treatment and controlled tests should be carried out if at all possible.)

3. Typical disease signs should be detectable in at least a few shrimp and the causative bacterial pathogen must be presumptively identified.
4. Since the efficacy of T200 medicated feed therapy for the control of mortality caused by bacterial pathogens is being tested, investigators must be prepared to make no changes in the shrimp cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct T200 medicated feed therapy. Complicating bacterial or other aquatic pathogens should be carefully documented. If necessary, these infections can be treated once T200 medicated feed response (efficacy) data has been collected. However, it may require as long as 10 days after the completion of T200 medicated feed therapy to determine differences between test and control groups and to complete post-treatment evaluations.

**Prior to initiating each treatment event**, the Investigator must first complete Form OTCS-W, "Worksheet for Designing Individual Field Trials" 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 (via 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. After initiation of the field trial, the Investigator should also record the assigned study number on Form OTCS-2a (and/or Form OTCS-2b) and Form OTCS-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 shrimp, or it may be individual animals.
- B. Separately confined, untreated control shrimp will not be required in supplementary field studies conducted to determine the effectiveness of T200 medicated feed treatment. Shrimp from a group or lot will first be examined to determine if treatment with T200 is required. When treatment is underway or has been completed, shrimp from the same group will be examined to determine the effect of treatment on the parameters used to initially sanction the treatment. Evaluation will in all cases consist of determining shrimp mortality, although in some cases degree or severity of bacterial infection may also be quantified.

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. Use of control groups will ensure that results of efficacy studies provide useful information that will support an NADA.

It is important that all shrimp are treated in a similar fashion. If shrimp 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 shrimp should be kept under conditions as similar as possible to treated shrimp for valid comparison. Although not required, replicate treatment groups are strongly encouraged in both treated and control groups. Assignment to control and treatment groups should be random and designed to avoid bias.

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 shrimp have been treated and which shrimp are controls.

The designation of specific treatment groups often depends upon the number of affected treatment units, the nature and severity of the disease being treated, and the variables being tested. Two or three different treatment groups are generally anticipated.

**1. Spotty, low level, or chronic disease patterns:**

Some facilities participating in this INAD are doing so as a means of being prepared, in advance, to use T200 medicated feed in the event a bacterial disease outbreak occurs. If management practices have been good, disease occurrences often result in low morbidity and mortality rates. T200 therapy may be occasionally required as a part of the process of a comprehensive shrimp health management program. These situations are the most typical. Even though there may be too few units involved to allow for treatment replication, careful record keeping is important so that useful data can be collected. Handling of clinically ill shrimp should be kept to a minimum until they have been successfully treated. Even the careful separation of diseased shrimp into new groups for treatment may alter environmental conditions present during disease initiation, thereby potentially rendering the T200 therapy trial meaningless.

**2. Epizootics:**

At some participating facilities disease outbreaks may be more widespread, more severe and occur more regularly. Sufficient shrimp and test units at these facilities may be available to conduct higher quality studies (i.e. studies that include replication, randomization, blinding, etc.). Such situations are suitable for the conduct of pivotal, carefully designed and controlled studies. Investigators at these facilities are encouraged to contact the AADAP Office for assistance with study design and completion. These facilities will be given top priority for the availability of treated feed, on-site assistance from the AADAP Office, and diagnostic support from shrimp health professionals.

## XI. TREATMENT SCHEDULES

### A. Route of administration

T200 will be administered only as a medicated feed treatment.

### B. Dosage and treatment duration

T200 will be administered in feed at a dosage of 4.5 g oxytetracycline per kg of feed, fed to satiation for 14 consecutive days.

### C. Dosing interval and repetition.

T200 will be administered as a single treatment regimen. A new treatment regimen may be initiated no sooner than five (5) days after the last treatment regimen ended.

### D. Drug preparation and administration procedures

T200 Medicated Feed Article will typically be incorporated into standard diets by an established feed manufacturer. However, in certain situations, T200 Medicated Feed Article may be top-coated on feed by investigators. Standard personal protective equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when preparing or administering T200 medicated feed. Medicated feed for each individual lot of shrimp should be accurately weighed prior to treatment. Shrimp should be fed in such a manner as to ensure optimal consumption of T200 medicated feed (see Feeding Regimen below).

### E. Feeding Regimen

The actual feeding regimen used will be left to the discretion of the investigator, and will likely be dictated by the feeding behavior of the shrimp. However, and as stated above in XI.B., shrimp should be fed TM200 medicated feed to satiation for 14 consecutive days. In all cases, the daily feeding regimen should be designed to maximize consumption of the medicated feed to result in shrimp receiving an adequate target dosage.

Specify on source data sheets how shrimp were fed (e.g., by hand, using automatic feeders, or feeding trays), amount of feed offered (i.e., % body weight per day), and whether feed was well accepted or poorly utilized.

### 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 make no changes in shrimp cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct T200 medicated feed treatment. However, if concomitant therapy is required in order to protect valuable shrimp stocks, it should be fully documented and the efficacy data from the T200 medicated feed treatment involved should be appropriately labeled.

## XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable shrimp based on hatchery records or other pertinent species information indicating 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 OTCS-3. Treatment response parameters that should be addressed include the following:

### 1. Primary Parameters

Control of Bacterial Diseases - Morbidity and mortality data, coupled with case history and analyses of bacterial load, usually indicate when T200 medicated feed treatment is needed. **Typically, source data must be collected for at least 5 days before treatment, during treatment, and for at least 10 days after the treatment period (i.e., the post-treatment period) has ended.** Collection of this data is critically important. Samples of hepatopancreas or other typically affected tissue will be removed from groups of representative shrimp and tested by bacteriological, serological, or other methods to determine the presence of target pathogens.

### 2. Secondary Parameters

Secondary parameters may also include general observations on shrimp behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative shrimp 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 extremely negative responses/behavior by the shrimp or hazards to the applicator. Although oxytetracycline medicated feed has been used extensively for many years with beneficial effect in shrimp culture, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species of shrimp. Carefully observe all treated shrimp 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 T200 medicated feed INAD 8069 will need to complete the following forms:

Form OTCS-W.	Worksheet for Designing Individual Field Trials under INAD 8069
Form OTCS-1.	Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
Form OTCS-2a.	Chemical Use Log for Clinical Field Trials Using Terramycin® 200 Medicated Feed under INAD 8069 - <u>Terramycin® 200 for Fish Type A Medicated Article</u>
Form OTCS-2b.	Chemical Use Log for Clinical Field Trials Using Terramycin® 200 Medicated Feed under INAD 8069 - <u>Terramycin® 200 for Fish Medicated feed</u>
Form OTCS-3.	Results Report Form for use of Terramycin® 200 for Fish under INAD 8069

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. All shrimp treated with T200 medicated feed must be maintained in culture facilities for a specified withdrawal time following completion of therapy before stocking/release or harvest. The investigational withdrawal period for shrimp being treated under INAD 8069 is 2 days.

No withdrawal period shall be required for shrimp that died during or after treatment that will be buried or rendered into non-edible products.

The Investigator must verify compliance with requirements regarding the disposition of all treated shrimp on Form OTCS-3.

## **XVI. DISPOSITION OF INVESTIGATIONAL DRUG**

T200 medicated feed will be used only in the manner and by the individuals specified in the Study Protocol. If any unused T200 medicated feed remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Spoiled or out-dated medicated feed should be disposed of in a landfill. 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 6) for information and details.

### **B. Study Monitors**

Study Monitors are generally shrimp health professionals with experience in diagnosing and treating shrimp diseases, and the ability to monitor overall shrimp health with respect to ongoing shrimp culture practices. A study monitor should be assigned to each facility that is authorized to treat shrimp with T200 medicated feed. 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 T200 medicated feed itself) are already available at each participating shrimp hatchery. The use of various drugs, chemicals, and therapeutants to meet management and/or production goals is a common occurrence at many shrimp hatcheries. Shrimp hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (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**

T200 medicated feed will be administered directly by the assigned Investigator (shrimp hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). T200 medicated feed will be maintained in a secure location, and only the Investigator or persons under his/her direct supervision will have access.

### **E. Drug accountability records**

See Section VII.A.6. Accountability (page 6) for details and Forms OTCS-W, OTCS-1, OTCS-2a, OTCS-2b, OTCS-3 (pages 12) 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 review the information and ensure that all required data 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.

## 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 OTCS-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. A copy of Form OTCS-2a (and/or Form OTCS-2b) should be sent to Study Monitors with the corresponding Form OTCS-3. A copy of Form OTCS-3 should be sent to the Study Monitor after completion of the entire treatment period, which includes the post-treatment observation period. **All forms must be submitted by the end of the calendar year.** 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.

## 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 report will be prepared and submitted to the FDA. This submission will probably include a request for an extension of the INAD based on the data collected during that year and whether or not sufficient shrimp remain within the slaughter authorization. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

## XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and 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.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with Form OTCS-3 and ultimately be submitted to the Study Director.