STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR OXYTETRACYCLINE (TERRAMYCIN 343®) FOR IMMERSION THERAPY (INAD #9033)

Sponsor:

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

_____ _____

Sponsor Signature Date Approved

Manufacturer:

Pfizer Animal Health
700 Portage Road, RIC-190-43
Kalamazoo, MI 49001-0199

Facility for Coordination of Terramycin 343® INAD:

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

Proposed Starting Date: July 1, 2007

Proposed Ending Date: June 30, 2012

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 343® IMMERSION THERAPY UNDER INAD #9033

I. STUDY ID AND TITLE:

Clinical field trials to determine the efficacy of Terramycin 343® immersion therapy to control mortality caused by certain bacterial diseases in cultured fish.

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

Manufacturer: Pfizer Animal Health
700 Portage Road
RIC-190-43
Kalamazoo, MI 49001-0199

Contact: Mark Subramanyam
Phone: 269-833-3388
Fax: 269-833-2707
Email: mark.subramanyam@pfizer.com

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

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:

Reauthorization Starting Date:	July 1, 2007
Reauthorization Expiration Date:	June 30, 2012
Proposed Termination Date:	To be determined by research progress.

V. BACKGROUND/PURPOSE:

Oxytetracycline has historically been the drug of choice when diagnostic evidence shows salmonids to have furunculosis, caused by *Aeromonas salmonicida*; bacterial hemorrhagic septicemia, caused by *Aeromonas (liquefaciens) hydrophila* and other closely related bacteria; pseudomonas disease, caused by *Pseudomonas sp.*; enteric redmouth, caused by *Yersinia ruckeri*; flexibacteriosis, caused by *Flexibacter (Cytophaga) columnaris, Flexibacter (Cytophaga) psychrophilus*, or closely related yellow pigmented gliding bacteria as described in U. S. Food and Drug Administration (FDA) Public Master File #5456; or, vibriosis caused by *Vibrio anguillarum, Vibrio ordalli* or other closely related bacteria.

In warmwater fish culture, oxytetracycline also has been useful in the control of enteric septicemia of catfish, caused by *Edwardsiella ictaluri* and bacterial hemorrhagic septicemia, pseudomonas disease, and flexibacteriosis in catfish, sturgeon, temperate basses, sunfishes, and other fish species including several listed as threatened or endangered under the Endangered Species Act.

Integrated fish health management practices usually prevent the occurrence of these diseases. However, adverse environmental conditions, uncontrollable water supplies and unforeseen factors can lead to severe disease outbreaks requiring prompt treatment in order to prevent significant losses of fish valuable to natural resource stewardship. 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 343®) in fish shall be designed to meet the needs of each species or lot, the size and numbers of fish 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 fish health, fish quality and survival, and to fully meet fishery management needs.

The purpose of this INAD is to develop clinical field trial data that will demonstrate the efficacy and safety of Terramycin 343® to control mortality caused by certain bacterial diseases in cultured fish under a variety of environmental conditions, at a wide range of temperatures, and in a variety of fish species. These data will be used to support a new animal drug application (NADA) for Terramycin 343®. Because there are many factors that can affect the success or failure of Terramycin 343® 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 should 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 extend and expand the current label for Terramycin 343® to cover major aquaculture needs. Therefore, the USFWS may request that the U. S. Food and Drug Administration (FDA) grant re-authorization of this Terramycin 343® 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 New Animal Drug Application (NADA) research data to support expanded labels claims for Terramycin 343®. 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 Terramycin 343® 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 Terramycin 343® immersion therapy to control mortality caused by certain bacterial diseases in cultured fish under a variety of environmental conditions, at a wide range of temperatures, and in a variety of fish species.

2. Provide an opportunity for fish culturists to legally use Terramycin 343® immersion therapy to control certain bacterial diseases in cultured fish that occur under a variety of environmental conditions, at a wide range of temperatures, and in a variety of fish species so that they can maintain healthy stocks of fish during the period of time necessary for collection of data that will be used to support expanded label claims for the use of Terramycin 343®.

Within these two relatively broad objective areas, there are also four more specific study protocol objectives. The four specific study objectives are described below:

Objective A

Determine the efficacy of a **single** bath treatment of 20 mg oxytetracycline per liter of water for 1 hour for the control of furunculosis, bacterial hemorrhagic septicemia, enteric redmouth, flexibacteriosis, and vibriosis in a variety of salmonid fish species under a variety of rearing or environmental conditions. Fish species that may be treated are listed in Appendix VIa. Treated fish must be maintained in culture facilities for a minimum of 21 days before they can be released or harvested.

Objective B

Determine the efficacy of a **single** bath treatment of 20 mg of oxytetracycline per liter of water for 1 hour for the control of enteric septicemia in catfish, and bacterial hemorrhagic septicemia, pseudomonas disease, and flexibacteriosis in catfish, sturgeon, temperate bass, and other cool and warmwater fish species listed in Appendix VIa. Treated fish must be maintained in culture facilities for a minimum of 21 days before they can be released or harvested.

Objective C

Determine the efficacy of bath treatment at 20 mg of oxytetracycline per liter of water for 1 hour administered on 1 to 4 consecutive days for the control of furunculosis, bacterial hemorrhagic septicemia, enteric redmouth, flexibacteriosis, and vibriosis in a variety of salmonid fish species under a variety of rearing or environmental conditions. Fish species that may be treated are listed in Appendix VIa. Treated fish must be maintained in culture facilities for a minimum of 60 days before they can be released or harvested.

Objective D

Determine the efficacy of bath treatment at 20 mg of oxytetracycline per liter of water for 1 hour administered on 1 to 4 consecutive days for the control of enteric septicemia in catfish, and bacterial hemorrhagic septicemia, pseudomonas disease, and flexibacteriosis in catfish, sturgeon, temperate bass, and other cool and warmwater fish species listed in Appendix VIa. Treated fish must be maintained in culture facilities for a minimum of 60 days before they can be released or harvested.

VII. MATERIALS:

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Common Name: Oxytetracycline hydrochloride

Product Name:: **Terramycin 343® Soluble Powder**

Chemical Family: Tetracycline derivative

CAS Number: 2058-46-0

Appearance: Yellow powder

Odor: None

b. Strength and dosage form

Terramycin 343® is a broad-spectrum, highly concentrated antibiotic powder intended for administration in the drinking water of swine for the control of specific diseases. It is also approved for use as an immersion treatment to mark the skeletal tissue of finfish fry and fingerlings (NADA 008-622). **Terramycin 343® contains 343g of active oxytetracycline hydrochloride per pound of product.**

c. Manufacturer, source of supply

Pfizer Animal Health
700 Portage Road, RIC-190-43
Kalamazoo, MI 49001-0199

Contact: Mark Subramanyam
Phone: 269-833-3388
Fax: 269-833-2707
Email: mark.subramanyam@pfizer.com.

2. Verification of drug integrity/strength:

The Manufacturer, Pfizer Animal Health, will provide the analytical data necessary to establish the purity of each lot of Terramycin 343® supplied. The lot number and date of manufacture for each batch of Terramycin 343® 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 OTIMM-1) will clearly identify the lot number and date of manufacture of Terramycin 343® shipments. If the integrity of the Terramycin 343® 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 OTIMM-2). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

Terramycin 343® must be stored 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 Terramycin 343® must be labeled to indicate that it contains hazardous material and that "*NO Food or Drink is to be Stored in this unit*".

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for Terramycin 343® (see Appendix IV). Each person involved with the study and each person who may be present during the use of Terramycin 343® shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with Terramycin 343® .

5. Investigational labeling

Copies of the labels to be attached to each container of Terramycin 343® are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of Terramycin 343®.

6. Accountability

Pfizer Animal Health will be the sole supplier of Terramycin 343® to all Investigators under INAD 9033.

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of Terramycin 343®, the Investigator will complete Form OTIMM-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 OTIMM-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 OTIMM-1 to FDA. Arrangements

should be made between Investigators and Study Monitors to insure completed Form OTIMM-1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of Terramycin 343® on-hand. A Chemical Use Log (Forms OTIMM-2) will be supplied to each Investigator. Each time Terramycin 343® is used, it must be recorded by the Investigator on Form OTIMM-2.

7. Preparation Procedures

Oxytetracycline will be supplied to Investigators as Terramycin 343® containing 343g of active oxytetracycline hydrochloride per pound of product. Hence, when calculating desired treatment concentration, Investigators should consider Terramycin 343® to be 75.6% active oxytetracycline. Terramycin 343® should not be adulterated in any manner prior to use.

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

Sampling techniques and diagnostic equipment will most likely be provided by trained fish health biologists 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 fish 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 Terramycin 343® INAD 9033 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 fish. This will generally be a groups of fish 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 Terramycin 343® 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.) is 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 a Form OTIMM-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).

V. Pathogen/disease considerations

A. Bacterial fish pathogens should be presumptively identified by procedures described in Section 3 of the "Blue Book" (Procedures for the Detection and Identification of Certain Fish Pathogens, Third Edition, Fish Health Section/American Fisheries Society, 1985). Other, more sensitive methods described elsewhere in peer-reviewed references, or as mutually determined by the local fish health biologist, in consultation with the Study Monitor, also may be used. (**Note**: **Diagnostic methods other than those in the Third Edition of the "Blue Book" should be described on a separate sheet attached to a Form OTIMM-3 "Results Report Form"**).

B. There should be increased mortality rates among fish in a rearing unit(s) for three or more consecutive days. (Note: Station history and the experience of the investigator, monitor, or the fish health biologist may over-ride 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.)

C. Typical disease signs should be detectable in at least a few fish and the causative pathogen should be identified.

D. Since the efficacy of Terramycin 343® immersion therapy for the control of mortality caused by bacterial diseases is being tested, investigators must be prepared to make no changes in the fish cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct Terramycin 343® therapy. Complicating bacterial or other aquatic pathogens should be carefully documented. If necessary, these infections can be treated once Terramycin 343® response (efficacy) data has been collected. However, it may require as long as 10 days after the completion of Terramycin 343® 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 OTIMM-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 (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 OTIMM-2 and Form OTIMM-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. Separately confined, untreated control fish will not be required in supplementary field studies conducted to determine the effectiveness of Terramycin 343® treatment. Fish from a group or lot will first be examined to determine if treatment with Terramycin 343® is required. When treatment is underway or has been completed, fish 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 fish mortality, although in some cases degree or severity of bacterial infestation 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 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. 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 fish have been treated and which fish 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:**

A number of facilities participating in this INAD are doing so as a means of being prepared, in advance, to use Terramycin 343® in the event a bacterial disease outbreak occurs. If management practices have been good, disease occurrences often result in low morbidity and mortality rates. Terramycin 343® therapy may be occasionally required as a part of the process of a comprehensive fish 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 fish should be kept to a minimum until they have been successfully treated. Even the careful separation of diseased fish into new groups for treatment may alter environmental conditions present during disease initiation, thereby potentially rendering the Terramycin 343®therapy trial meaningless.

2. **Epizootics:**

At some participating facilities disease outbreaks may be more widespread, more severe, and occur more regularly. Sufficient fish 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 assistance in study design, on-site assistance from the AADAP Office, and diagnostic support from fish health biologists.

XI. TREATMENT SCHEDULES

A. Route of administration

Terramycin343® will be administered only as an immersion treatment.

B. Dosage, treatment duration, and dosing interval/repetition

Objective A [For the control of furunculosis, bacterial hemorrhagic septicemia, enteric redmouth, flexibacteriosis, and vibriosis in a variety of salmonid fish species]

Treatment at 20 mg oxytetracycline per liter of water for 1 hour.

A single treatment event with no repetition.

Objective B [For the control of enteric septicemia in catfish, and bacterial hemorrhagic septicemia, pseudomonas disease, and flexibacteriosis in catfish, sturgeon, temperate bass, and other cool and warmwater fish species]

Treatment at 20 mg oxytetracycline per liter of water for 1 hour.

A single treatment event with no repetition.

Objective C [For the control of furunculosis, bacterial hemorrhagic septicemia, enteric redmouth, flexibacteriosis, and vibriosis in a variety of salmonid fish species]

Treatment at 20 mg oxytetracycline per liter of water for 1 hour.

One to four treatments administered on consecutive days.

Objective D [For the control of enteric septicemia in catfish, and bacterial hemorrhagic septicemia, pseudomonas disease, and flexibacteriosis in catfish, sturgeon, temperate bass, and other cool and warmwater fish species]

Treatment at 20 mg oxytetracycline per liter of water for 1 hour.

One to four treatments administered on consecutive days.

C. Drug preparation and administration procedures

Oxytetracycline will be supplied to Investigators as Terramycin 343® containing 343g of active oxytetracycline hydrochloride per pound of product. Hence, when calculating desired treatment concentration, Investigators should consider Terramycin 343® to be 75.6% active oxytetracycline. Terramycin 343® should be thoroughly mixed in rearing unit water. Terramycin 343® should not be adulterated in any manner prior to use.

D. 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 fish cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct Terramycin 343® treatment. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the Terramycin 343® 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 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 OTIMM-3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

   Morbidity and mortality data, coupled with case history and analyses of bacterial load, usually indicate when Terramycin 343® treatment is needed. **Typically, source data must be collected for 5 days before treatment, during treatment, and for 10 days after the treatment period has ended**. Collection of this data is critically important. Samples of kidney or other tissue should be removed from groups of representative fish 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 fish behavior and response to routine culture/handling activities. This 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 extremely negative responses/behavior by the fish or hazards to the applicator. Although Terramycin 343® immersion therapy has been used extensively for many years with beneficial effect in fish culture, 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 Terramycin 343® INAD 9033 will need to complete the following forms:

Form OTIMM-W.	Worksheet for Designing Individual Field Trials under INAD 9033
Form OTIMM-1.	Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
Form OTIMM-2.	Chemical Use Log for Clinical Field Trials Using Terramycin 343® under INAD 9033
Form OTIMM-3.	Results Report Form for use of Terramycin 343® under INAD 9033

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 fish treated with Terramycin 343® must be maintained in culture facilities for a specified withdrawal time following completion of therapy before stocking/release or harvest. Specific withdrawal time is based upon treatment objective as defined in Section XI.B of this study protocol and are as follows:

Objective A:	21 days
Objective B:	21 days
Objective C:	60 days
Objective D:	60 days

No withdrawal period will be required for stocked fish that will not be harvestable/catchable after release, or are illegal for harvest, during the withdrawal times specified above (e.g., if fish are treated under Objective C and are not susceptible to harvest for a minimum of 60 days following completion of treatment, they may be stocked/released immediately). No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

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

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Terramycin 343® will be used only in the manner and by the individuals specified in the Study Protocol. If any unused Terramycin 343® remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Spoiled or out-dated Terramycin 343® 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 5) for information and details.

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor should be assigned to each facility that is authorized to treat fish with Terramycin 343®. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. 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 Terramycin 343® itself are already available at each participating fish hatchery. 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 (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 6).

D. Administrator of the drug

Terramycin 343® will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Terramycin 343® 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 5) for details and Forms OTIMM-W, OTIMM-1, OTIMM-2, and OTIMM-3 (page 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 OTIMM-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. A copy of Form OTIMM-2 should be sent to Study Monitors with the corresponding Form OTIMM-3. A copy of Form OTIMM-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. 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 OTIMM-3, and ultimately be submitted to the Study Director.