

**STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL
NEW ANIMAL DRUG (INAD) EXEMPTION FOR OXYTETRACYCLINE
(TERRAMYCIN® 200 for Fish) MEDICATED FEED (INAD #9332)**

Sponsor:

U.S. Fish and 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® 200 for Fish INAD:

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

Proposed Starting Date: April 1, 2008

Proposed Ending Date: March 31, 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® 200 FOR FISH MEDICATED FEED UNDER INAD #9332

I. STUDY ID AND TITLE:

Clinical field trials to determine the efficacy of Terramycin® 200 for Fish when fed as a medicated feed to 1) control mortality caused by bacterial diseases in a variety of freshwater and marine fish species; and 2) mark skeletal tissue in a variety of freshwater and marine fish species. [Note: No clinical field trials will be conducted under this INAD for use patterns for which Terramycin® 200 has already received FDA-approval (e.g., treatment of coldwater disease in freshwater-reared salmonids and treatment of columnaris in *Oncorhynchus mykiss* (NADA 038-439)].

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: Phibro Animal Health
710 Route 46 East, Suite 401
Fairfield, NJ 07004.

Contact: Paul Duquette
Phone: 973-575-5255
Fax: 973-575-4354
Email: paul.duquette@pahc.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: April 1, 2008

Reauthorization Expiration Date: March 31, 2012

Proposed Termination Date: To be determined by research progress.

V. BACKGROUND/PURPOSE:

In addition to the use patterns listed on the current label, Oxytetracycline historically has often been the drug of choice when diagnostic evidence shows salmonids to have enteric redmouth (ERM) 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 Public Master File #5456; or, vibriosis caused by *Vibrio anguillarum*, *Vibrio ordalli* or other closely related bacteria. Oxytetracycline also has been useful for the control of bacterial hemorrhagic septicemia caused by *Aeromonas hydrophila* and other closely related bacteria, pseudomonas disease caused by *Pseudomonas* sp., or flexibacteriosis in several other families of fishes including sturgeons, pikes, sunfishes (bass), and perches.

In recent years, studies have shown evidence that Oxytetracycline may be effective in controlling bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* (John Cvitanich, 1995 personal communication). Additional clinical field trials are needed to follow up on this lead.

Integrated fish health management practices usually prevent the occurrence of these diseases. However, adverse environmental conditions, physiological changes related to smoltification or the onset of spawning, uncontrollable water conditions, and unforeseen factors can lead to severe disease outbreaks requiring prompt treatment to prevent significant losses in excess of 50 percent of fish in public, tribal and private aquaculture. 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® 200 for Fish) 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 or aquaculture objectives. Because there are many factors that can affect the success or failure of Terramycin® 200 for Fish therapy, data is needed to determine the best ways to use the drug to obtain effective disease control enroute 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 Terramycin® 200 for Fish therapy to control mortality caused by bacterial diseases of freshwater and marine fish that may occur in a variety of environmental conditions, at a wide range of temperatures, and in a variety of cultured fish species. Specifically, the objective of clinical field efficacy trials is to evaluate the efficacy of Terramycin® 200 for Fish medicated feed treatment to control mortality in a variety of fish species caused by diseases susceptible to oxytetracycline. Efficacy trials will be conducted at a number of different study sites, on a variety of fish species infected with a variety of fish pathogens. Diseases of interest include, but are not limited to: 1) coldwater disease; 2) columnaris; 3) furunculosis, 4) enteric septicemia in catfish; 5) enteric redmouth; 6) vibriosis, and 7) bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads. Another purpose of this INAD exemption is to obtain additional clinical field trial data to demonstrate the efficacy and target animal safety of Terramycin® 200 for Fish therapy to mark skeletal tissue in a variety of freshwater and marine fish species.

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 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® 200 for Fish medicated feed 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® 200 for Fish. 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® 200 for Fish 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® 200 for Fish to 1) control certain bacterial diseases of freshwater and marine fish that occur under a variety of environmental conditions, at a wide range of temperatures, and in a variety of cultured fish species; and 2) mark skeletal tissue in a variety of freshwater and marine fish species. **[Note: No clinical field trials will be conducted under this INAD for use patterns for which Terramycin® 200 has already received FDA-approval (e.g., treatment of coldwater disease in freshwater-reared salmonids and treatment of columnaris in *Oncorhynchus mykiss* (NADA 038-439)].**
2. Provide an opportunity for fish culturists to legally use Terramycin® 200 for Fish medicated feed to control certain bacterial diseases of fish that occur under a variety of environmental conditions, at a wide range of temperatures, and in a variety of cultured 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® 200 for Fish on freshwater and marine fish.

Specific study objectives of this study protocol are as follows:

Objective A

Determine if the oxytetracycline dosage and duration, for which the drug already has been labeled for the control of furunculosis in salmonids (2.5 - 3.75 grams of active drug per 100 pounds of fish per day for 10 days (i.e., "the standard dosage and duration") is efficacious and safe when fed to fish for the control of mortality caused by coldwater disease, columnaris, flexibacteriosis, enteric redmouth, bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads, and other gram negative systemic bacteria in a variety of salmonid species (see Appendix VIa), under a variety of rearing or environmental conditions. **Salmonid fish treated as specified in Objective A may be released for immediate harvest or slaughtered for processing after a 21-day withdrawal period.** No withdrawal period is required for fish that will not be catchable for 21 or more days after release or are illegal for harvest.

Objective B

Determine the efficacy and target animal safety of a dosage of 10.0 grams of active oxytetracycline per 100 pounds of fish per day for 14 days for the control of mortality caused by deep-seated bacterial infections in a variety of freshwater and marine fish species (see Appendix VIa). Treated fish will be reared under a variety of environmental conditions at water temperatures not below 4°C. However, please note that treatment may not be administered to fish in net pens. **A withdrawal period of 70 days is required for all species treated as described in Objective B** until research studies demonstrate that a shorter withdrawal period meets FDA requirements for the release or slaughter of treated fish. No withdrawal period is required for fish that will not be catchable for 70 or more days after release or are illegal for harvest.

Objective C

Determine the efficacy of the "standard dosage and duration" of oxytetracycline therapy (as described in Objective A) for the control of mortality caused by a variety of bacterial pathogens sensitive to oxytetracycline in a variety of non-salmonid freshwater and marine fish species (see Appendix VIa) reared at a variety of water temperatures and under a variety of rearing or environmental conditions. However, please note that treatment may not be administered to fish in net pens. Because little data is available, **a withdrawal period of 40 days is required for fish treated in clinical field trials under Objective C** until research studies demonstrate that a shorter withdrawal period meets FDA requirements for the release or slaughter of treated fish. No withdrawal period is required for fish that will not be catchable for 40 or more days after release or are illegal for harvest.

Objective D

Determine the efficacy of oxytetracycline treatment at up to 6.0 g active oxytetracycline per 100 lbs body weight per day for 14 days to control mortality caused by **withering syndrome in abalone**. Based on data submitted to FDA, **a withdrawal period of 35 days will be required before abalone can be released or harvested**. No withdrawal period is required for abalone that will not be harvestable for 35 or more days after release, or are illegal for harvest.

Objective E

Determine the efficacy of oxytetracycline at the standard dosage and duration or at 10.0 grams of active oxytetracycline per 100 pounds of fish per day for 14 days (high dose) **to mark skeletal tissue in a variety of freshwater and marine fish species. Salmonid fish treated as specified in the standard dosage may be released for immediate harvest or slaughtered for processing after a 21-day withdrawal period.** No withdrawal period is required for fish that will not be catchable for 21 or more days after release or are illegal for harvest. **Non-salmonid fish treated as specified in the standard dosage may be released for immediate harvest or slaughtered for processing after a 40-day withdrawal period treated in clinical field trials under Objective F.** No withdrawal period is required for fish that will not be catchable for 40 or more days after release or are illegal for harvest. **A withdrawal period of 70 days is required for all fish species treated at the high**

dose until research studies demonstrate that a shorter withdrawal period meets FDA requirements for the release or slaughter of treated fish. No withdrawal period is required for fish that will not be catchable for 70 or more days after release or are illegal for harvest.

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)

Chemical Family: Tetracycline derivative

CAS Number: 79-57-2

Appearance: Uniform tan meal

Odor: Cereal odor

b. Strength and dosage form

Terramycin® 200 for Fish is a broad-spectrum anti-infective with a specially designed formula for fish. It has been proven highly effective in controlling diseases caused by Gram-positive and Gram-negative organisms that adversely affect salmonids, catfish, and lobsters. **Terramycin® 200 for Fish 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: 973-575-5255
Fax: 973-575-4354
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 Terramycin® 200 for Fish Type A Medicated Article supplied. The lot number and date of manufacture for each batch of Terramycin® 200 for Fish 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 OTC-1) will clearly identify the lot number and date of manufacture of Terramycin® 200 for Fish shipments (i.e., Type A Medicated Article or medicated feed). If the integrity of the Terramycin® 200 for Fish 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 OTC-2a and/or Form OTC-2b). The Study Monitor assigned to the Investigator involved will be immediately notified.

Based on discussions with Investigators concerning planned feed rate and kg of fish to be medicated, commercial fish feed manufacturers shall prepare feed with dosages of Terramycin® 200 for Fish to assure the desired target dose is achieved (see Sections VI and XI for dosage options).

The Investigator may also prepare his/her own drug-treated feed by top-coating feed on-hand (or specially ordered feed) with Terramycin® 200 for Fish. If the Investigator chooses this option, they are encouraged (but not required) to have a sample of the top-coated feed assayed for oxytetracycline concentration by a certified, analytical testing laboratory. Results of drug-treated feed assays should be appended to a Form OTC-3.

3. Storage Conditions

Terramycin® 200 for Fish Type A Medicated Article 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® 200 for Fish 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*". Terramycin® 200 for Fish medicated feed should be stored at temperatures and for periods of time not to exceed limits set by the feed manufacturer. 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 Terramycin® 200 for Fish Type A Medicated Article (see Appendix IV). Each person involved with the study and each person who may be present during the use of Terramycin® 200 for Fish 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 Terramycin® 200 for Fish.

5. Investigational labeling

Copies of the labels to be attached to each container of Terramycin® 200 for Fish Type A Medicated Article and all bags of Terramycin® 200 for Fish medicated feed are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of Terramycin® 200 for Fish Type A Medicated Article and Terramycin® 200 for Fish medicated feed.

6. Accountability

Phibro Animal Health will be the sole supplier of Terramycin® 200 for Fish Type A Medicated Article to all Investigators under INAD 9332.

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of Terramycin® 200 for Fish Type A Medicated Article or Terramycin® 200 for Fish medicated feed, the Investigator will complete Form OTC-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 OTC-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 OTC-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form OTC-1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of Terramycin® 200 for Fish Type A Medicated Article and/or Terramycin® 200 for Fish medicated feed on-hand. Chemical Use Logs (Forms OTC-2a and OTC-2b) will be supplied to each Investigator. Each time Terramycin® 200 for Fish Type A Medicated Article and/or Terramycin® 200 for Fish medicated feed is used, it must be recorded by the Investigator on Form OTC-2a and/or Form OTC-2b, respectively.

7. Preparation Procedures

Oxytetracycline will be supplied to Investigators either as Terramycin® 200 for Fish Type A Medicated Article or Terramycin® 200 for Fish medicated feed. Neither product should be adulterated in any manner prior to use. If Investigators are using Terramycin® 200 for Fish Type A Medicated Article to make their own oxytetracycline medicated feed, Terramycin® 200 for Fish 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 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.

2. Skeletal Marking

Sampling and diagnostic equipment should include standard dissecting equipment, as well as clean microscope slides, cover slips, and a fluorescent microscope. Generally, the procedures of Secor et al. (1991) should be used as a guide for sample processing and mark reading in clinical field trials conducted under this

INAD. Both fish capture equipment and fish sampling/diagnostic equipment are routinely available at hatcheries. Microscopes should be equipped with 100 watt fluorescent bulbs and filter sets for wave lengths in the 360 nm range to enable reading of otoliths with little or no polishing.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by Oxytetracycline INAD 9332 will need to complete several forms. These forms are described in Section XIII (p. 14). 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[®] 200 for Fish 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.) is presented in Appendix VIb and Appendix VIc.

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 OTC-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 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 OTC-3 "Results Report Form"**).

2. 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.)
3. Typical disease signs should be detectable in at least a few fish and the causative bacterial pathogen must be identified.
4. Since the efficacy of Terramycin® 200 for Fish 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 fish cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct Terramycin® 200 for Fish medicated feed therapy. Complicating bacterial or other aquatic pathogens should be carefully documented. If necessary, these infections can be treated once Terramycin® 200 for Fish medicated feed response (efficacy) data has been collected. However, it may require as long as 10 days after the completion of Terramycin® 200 for Fish 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 OTC-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 OTC-2a (and/or Form OTC-2b) and Form OTC-3b (or Form OTC-3c), 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® 200 for Fish medicated feed treatment. Fish from a group or lot will first be examined to determine if treatment

with Terramycin® 200 for Fish 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 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 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® 200 for Fish 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. Terramycin® 200 for Fish 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® 200 for Fish 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 the availability of treated feed, on-site assistance from the AADAP Office, and diagnostic support from fish health biologists.

XI. TREATMENT SCHEDULES

A. Route of administration

Terramycin® 200 for Fish will be administered only as a medicated feed treatment.

B. Dosage and treatment duration

Objective A [For the control of mortality caused by coldwater disease, columnaris, flexibacteriosis, enteric redmouth, bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads, and other gram negative systemic bacteria **in salmonid fish species**]

Treatment at 2.5 - 3.75 grams of active drug per 100 pounds of fish per day for 10 days.

Objective B [For the control of mortality caused by deep-seated bacterial infections **in a variety of freshwater and marine fish species**]

Treatment at 10 grams of active drug per 100 pounds of fish per day for 14 days.

Objective C [For the control of mortality caused by a variety of bacterial pathogens sensitive to oxytetracycline **in a variety of non-salmonid freshwater and marine fish species**]

Treatment at 2.5 - 3.75 grams of active drug per 100 pounds of fish per day for 10 days.

Objective D [For the control of mortality caused by **withering syndrome in abalone**]

Treatment at up to 6.0 g active oxytetracycline per 100 lbs body weight per day for 14 days.

Objective E [For the **marking of skeletal tissue in a variety of fish species**]

Treatment at 2.5 - 3.75 grams of active drug per 100 pounds of fish per day for 10 days (standard dose) **or** 10 grams of active drug per 100 pounds of fish per day for 14 days (high dose).

C. Dosing interval and repetition

Terramycin® 200 for Fish will be administered as a single treatment regime, with no repetition of treatment.

D. Drug preparation and administration procedures

Terramycin® 200 for Fish Medicated Feed Article will typically be incorporated into standard diets by an established feed manufacturer. However, in certain situations, Terramycin® 200 for Fish 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 Terramycin® 200 for Fish medicated feed. Medicated feed for each individual lot of fish should be accurately weighed prior to treatment. Fish should be fed in such a manner as to ensure optimal consumption of Terramycin® 200 for Fish medicated feed (see Feeding Regime below).

5. Feeding Regime

During the course of therapy fish may be fed only treated feed, or a combination of treated and untreated feed. The actual feeding regime used will be left to the discretion of the investigator, and will be dictated by the feeding behavior of the fish to be treated and level of premix incorporated in the feed. In most cases it is anticipated that use of only treated feed will work best. However, in some cases, treated feed followed by untreated feed may be determined to be the optimal feeding regime. In still other cases, a small amount of untreated feed followed by a “full course” of treated feed may be utilized. In all cases, the daily feeding regime should be designed to maximize consumption of the treated feed to result in fish receiving the target dosage.

Specify on source data sheets how fish were fed (e.g. % treated feed vs % untreated feed, by hand, using automatic feeders, utilizing demand feeders), amount of feed offered (% body weight), 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 fish cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct Terramycin® 200 for Fish medicated feed 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® 200 for Fish 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 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 OTC-3a, Form OTC3b, or form OTC-3c. 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 Terramycin® 200 for Fish medicated feed treatment is needed. **Typically, source data must be collected for at least 5 days before treatment, during treatment, and for up to at least 20 days after the treatment period has ended.** Collection of this data is critically important. Samples of kidney or other tissue will be removed from groups of representative fish and tested by bacteriological, serological, or other methods to determine the presence of target pathogens.

Skeletal Marking - Primary parameters include the efficacy of the marking procedure, mark retention data (if possible), and morbidity and mortality data related to the marking procedure. Fish should be evaluated for mark detection and mark quality immediately following treatment. When possible, fish that are retained on station for any significant period of time after marking should be evaluated a second time for mark detection and mark quality as close as possible to the time of release. Mark detection and quality should be determined using an ordinal scale (0, 1, 2, and 3) as described on the top of Form OTC-3c. A minimum of 15 fish should be individually examined during each evaluation. Although it will be at the discretion of individual investigators to determine which bony structure they choose to evaluate for mark detection and determination of mark quality (e.g. vertebrae or otolith), the selected structure(s) must be clearly identified on Form OTC-3c.

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 oxytetracycline medicated feed 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® 200 for Fish medicated feed INAD 9332 will need to complete the following forms:

Form OTC-W.	Worksheet for Designing Individual Field Trials under INAD 9332
Form OTC-1.	Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
Form OTC-2a.	Chemical Use Log for Clinical Field Trials Using Terramycin® 200 Medicated Feed under INAD 9332 - <u>Terramycin® 200 for Fish Type A Medicated Article</u>
Form OTC-2b.	Chemical Use Log for Clinical Field Trials Using Terramycin® 200 Medicated Feed under INAD 9332 - <u>Terramycin® 200 for Fish medicated feed</u>
Form OTC-3b	Results Report Form for use of Terramycin® 200 for Fish under INAD 9332 - <u>All use excluding salmonids with coldwater disease and Oncorhynchus mykiss with columnaris treated at 3.75 g/100 lb fish/day</u>
Form OTC-3c	Results Report Form for use of Terramycin® 200 for Fish under INAD 9332 - <u>For use in the marking of skeletal tissue</u>

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® 200 for Fish medicated feed 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: 70 days
- Objective C: 40 days
- Objective D: 35 days
- Objective E: 21 days for standard dose (salmonids); 40 days for standard dose (non-salmonids); 70 days for high dose (all species)

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 A and are not susceptible to harvest for a minimum of 21 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 OTC-3b or Form OTC-3c.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Terramycin® 200 for Fish medicated feed will be used only in the manner and by the individuals specified in the Study Protocol. If any unused Terramycin® 200 for Fish 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 7) 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® 200 for Fish medicated feed. 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® 200 for Fish medicated feed 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 8).

D. Administrator of the drug

Terramycin® 200 for Fish medicated feed will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Terramycin® 200 for Fish 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 7) for details and Forms OTC-W, OTC-1, OTC-2a, OTC-2b, OTC-3b, and OTC-3c (page 14) 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 OTC-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. A copy of Form OTC-2a (and/or Form OTC-2b) should be sent to Study Monitors with the corresponding Form OTC-3b (or Form OTC-3c). A copy of Form OTC-3b or Form OTC-3c 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 forwarded 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 OTC-3b and/or Form OTC-3c, and ultimately be submitted to the Study Director.