

**STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL
NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUAFLO[®]
(florfenicol) USE IN MEDICATED-FEED (INAD #12-061)**

[Note: INAD 12-061 *Only For Use in Lobsters*]

Sponsor:

U.S. Fish and Wildlife Service, Office of Fisheries

Sponsor Signature

Date Approved

Manufacturer:

Intervet/Schering-Plough Animal Health
556 Morris Avenue
Summit, NJ 07901

Facility for Coordination of Aquaflor[®] INAD 12-061:

Aquatic Animal Drug Approval Partnership Program
U.S. Fish and Wildlife Service
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date: August 1, 2011

Proposed Ending Date: July 31, 2015

Study Director: Mr. Jim Bowker (USFWS/AADAP)

Study Director Signature

Date

Clinical Field Trial Location and Trial Number:

Facility Name

Investigators Name

Investigator Signature

Date

**STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG
(INAD) EXEMPTION FOR AQUAFLO[®]R USE IN MEDICATED-FEED UNDER INAD #12-061**

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of Aquaflor[®] medicated-feed fed to lobsters in order to control mortality caused by bacterial pathogens. INAD #12-061.

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: Intervet/Schering-Plough Animal Health
556 Morris Avenue
Summit, NJ 07901

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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

Principal Clinical Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

Study Monitors for Aquaflor[®] INAD: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: August 1, 2011

Proposed Completion Date: July 31, 2015

V. BACKGROUND/PURPOSE

Florfenicol is a potent, broad spectrum antibacterial agent with bacteriostatic properties (Horsberg et al 1996). It is a fluorinated analogue of thiamphenicol, and is similar in structure to chloramphenicol. Both thiamphenicol and chloramphenicol have been used as broad spectrum veterinary antibiotics (Nagata and Oka 1996). Aquaflor[®] is an aquaculture premix containing the novel antibiotic, florfenicol. Aquaflor[®] is available only from Intervet/Schering-Plough Animal Health. For additional information on florfenicol and Aquaflor[®] see Addendum II.

Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Bjorndal 1990; Clarke and Scott 1989; Frefichs and Roberts 1989). While the importance of environmental conditions (Hastien 1988; McCarthy and Roberts 1980; Munro and Roberts 1989) and the value of effective vaccines, where available (Ellis 1989), are acknowledged, antimicrobial therapy presently has an important role to play in aquaculture (Alderman 1988; Klontz 1987).

Antibiotic therapy is also considered a primary tool for the control of certain bacterial diseases in crustaceans, most notably for the disease gaffkemia which was a major cause of mortality in lobsters held in tidal impoundments during the 1970s and 1980s (Basti et. al, 2011). More recently, the Gram-negative bacterium *Photobacterium indicum* has been cultured from over 95% of morbid and dead lobsters held in Maine tidal impoundments, and may represent a new/emerging threat to lobster culture. Preliminary in vitro susceptibility data indicate that *Photobacterium indicum* is susceptible to florfenicol treatment.

Aquaflor[®] is currently FDA-approved in the U.S for 1) the control of mortality due to enteric septicemia in catfish; 2) the control of mortality due to columnaris disease in catfish; 3) the control of mortality due to furunculosis in freshwater-reared salmonids; and 4) the control of mortality due to coldwater disease in freshwater-reared salmonids. Aquaflor[®] is also approved in Canada for the control of furunculosis in Atlantic salmon. Not surprisingly, Aquaflor[®] has become a strong candidate for other potential approved uses in U.S. aquaculture, including use to control mortality caused by bacterial diseases in cultured/captive lobsters.

The objective of these field based clinical efficacy trials is to evaluate the efficacy of Aquaflor[®] medicated feed treatment to control mortality in lobsters caused by bacterial pathogens susceptible to florfenicol. Efficacy trials will be conducted at a number of different study sites. Although the primary bacterial pathogen of interest is *Photobacterium indicum*, clinical efficacy trials may also be conducted on other bacterial pathogens susceptible to florfenicol.

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 establish the effectiveness of Aquaflor[®] medicated-feed to control certain bacterial diseases of cultured lobsters that occur in a variety of environmental conditions and at a wide range of temperatures.
2. Provide an opportunity for lobster culturists to legally use Aquaflor[®] medicated-feed to control certain bacterial diseases of lobsters that occur in a variety of environmental conditions, at a wide range of temperatures, and in a variety of cultured lobster species so that they can maintain healthy stocks of lobster during

the period of time necessary for collection of data that will be used to support a New Animal Drug Application for the use of Aquaflor[®] in various lobster species.

Specific study objectives are described below:

Objective A

Determine if the Aquaflor[®] use pattern for which the drug has already been labeled in the U.S. for the control of specific bacterial pathogens in specific freshwater-reared fish species (i.e., **10 mg of florfenicol per kg body weight per day for 10 consecutive days**) is efficacious when used to control of mortality caused by bacterial pathogens in lobsters. Lobsters treated in this manner may be released for immediate harvest after a **21-day withdrawal period** (from the date of last treatment). No withdrawal period will be required for lobsters that will not be catchable during the above-described withdrawal periods, or are illegal for harvest during those periods.

Objective B

Determine if Aquaflor[®] treatment at a dosage of **15 mg of florfenicol per kg body weight per day for 10 consecutive days** is efficacious (or in some cases possibly more efficacious than treatment at a dosage of 10 mg florfenicol per kg body weight per day for 10 consecutive days) when used to control mortality caused by bacterial pathogens in lobsters. Lobsters treated in this manner may be released for immediate harvest after a **28-day withdrawal period** (from the date of last treatment). No withdrawal period will be required for lobsters that will not be catchable during the above-described withdrawal periods, or are illegal for harvest during those periods.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Intervet/Schering-Plough Animal Health's Aquaflor[®] Type A Medicated Article (antibiotic premix) containing 500 grams of florfenicol per kg of premix will be the only form of the drug used by feed manufacturers to formulate medicated-feed.

b. Chemical name - active component(s)

D-(threo)-1-(p-methylsulfonylphenyl)-2-dichloroacetamide-3-fluoro-1-propanol. This is the final formula. Florfenicol is a pure compound with no inactive ingredients.

c. Molecular formula

$C_{12}H_{14}NO_4C_{12}FS$

d. Molecular weight

358.20

e. Appearance and odor

White amorphous lumpy powder

f. Strength and dosage form

Drug concentration in the diet and feeding regimes will be designed to provide a daily dosage of either 10 or 15 mg of florfenicol per kg of fish.

g. Manufacturer, source of supply

Intervet/Schering-Plough Animal Health
556 Morris Avenue
Summit, NJ 07901

Contact person: Dr. Richard Endris, Research Program Manager
Telephone: (908) 473-5618
Fax: (908) 473-5560

2. Verification of Drug Integrity/Strength

Intervet/Schering-Plough Animal Health will provide limited analytical support in the event questions arise regarding product quality and drug activity. Presently, no provisions are in place to assay medicated feed used in supplemental efficacy trials. However, medicated feed used in pivotal efficacy trials will be assayed to verify drug integrity/strength. Investigators must record treated feed lot number, or chemical lot number of premix if top-coating, on Form FFCL-1 Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals.

Based on discussions with Investigators concerning planned feed rate and kg of lobsters to be medicated, commercial feed manufacturers shall prepare feed with concentrations of Aquaflor[®] premix to assure that target dosages of either 10 or 15 mg florfenicol/kg lobster/day are achieved.

3. Storage Conditions

Treated feed will be stored at temperatures and for periods of time not to exceed limits set by the feed manufacturer. Treated feed should be ordered only as needed and not stored for possible future use.

Aquaflor[®] premix should be stored at temperatures and for periods of time not to exceed the limits set by Intervet/Schering-Plough Animal Health. Inventories of both treated

feed and premix on-hand must be recorded on either Form FFCL-2a or Form FFCL-2b Chemical Use Log for Aquaflor®.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for Aquaflor® (Appendix IV). Each person involved with the study and each person who may be present during the use of Aquaflor® shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with Aquaflor®. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., should be worn at all times.

The possible hazards associated with the handling of Aquaflor® treated feed should be discussed, at least once per year, at station Safety meetings. Individuals with known allergic reactions to florfenicol (i.e. Aquaflor®) will not be permitted to handle such feed. For handling incidents or transportation emergencies telephone CHEMTREC, 800/424-9300.

5. Investigational labeling

Copies of the labels to be attached to each sachet of Aquaflor® and/or bag of Aquaflor® medicated-feed are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all bags of medicated-feed.

6. Accountability

1. USFWS Facilities and Non-USFWS Facilities

Immediately upon receiving an order/shipment of Aquaflor® medicated-feed or Aquaflor® premix, the Investigator must complete Form FFCL-1 "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The Investigator will archive the original in the facility's INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form FFCL-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 FFCL-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form FFCL-1s are received by the Study Director in a timely manner.

Investigators are also responsible for maintaining an accurate inventory of Aquaflor® premix and/or Aquaflor® medicated-feed on hand. Chemical Use Logs (Form FFCL-2a and Form FFCL-2b) will be supplied to each Investigator. Each time Aquaflor® premix and/or Aquaflor® treated feed is used, it must be reported by the Investigator on either Form FFCL-2a or Form FFCL-2b, respectively.

B. Items Needed for Sample Collection, Observations, Etc.:

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

VIII. EXPERIMENTAL UNIT

The experimental unit in these clinical field trials will consist of contained or isolated groups of lobsters. This could be groups of lobsters contained in tanks, raceways, ponds, or pounds.

IX. ENTRANCE CRITERIA

Bacterial pathogens should be presumptively identified prior to the initiation of treatment. The suggested protocol for bacteriological workup in lobsters is as follows: 1) the dorsal carapace of a morbid lobster is disinfected with 70% ethanol; 2) hemolymph is collected from the dorsal abdominal sinus with a sterile needle and syringe; 3) 0.2 ml of hemolymph is streaked onto a Trypticase Soy Agar (TSA) plate with 5% sheep red blood cells supplemented with 1.5 % NaCl or a Marine Sea Salt Agar (MSSA) plate; and 4) plates are incubated at 16° C for 48-72 hrs. At the end of the incubation period, predominant bacterial colonies are selected from the plates and preliminary biochemical profiling is conducted utilizing Gram stain, 0129 vibriostat susceptibility (150 mg), oxidase or catalase reaction, and carbohydrate utilization and H₂S production with Triple Sugar Iron (TSI) media. Final bacterial identification is by DNA sequence analysis and the Biolog® Microbial Identification System (Basti et al., 2010. Factors affecting post-capture survivability of lobster *Homarus americanus*. Diseases of Aquatic Organisms 90:153-166). Other methods described elsewhere in peer-reviewed references, or as mutually determined by the local aquatic animal health biologist, in consultation with the Study Monitor, may also be used. **(Note: Diagnostic methods other than those outlined above should be described on a separate sheet attached to Form FFCL-3 Diagnosis, Treatment, and Mortality Record).**

Other entrance criteria for the use of Aquaflor® medicated-feed are as follows:

1. The proposed facility and the investigator must be listed in Appendix IIIa of this Study Protocol before drug-treated feed can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, the Study Director, or by an Investigator to control emergency disease outbreaks (See Section XX).
2. There should be increased mortality rates among lobsters in a rearing unit(s) for three or more consecutive days. **(Note: Station history and the experience of the investigator, monitor, or the aquatic animal 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 lobsters and the causative bacterial agent must be identified.
4. Since the efficacy of Aquaflor® therapy for the control of a specific disease is being tested, Investigators must be prepared to make no changes in culture procedures or environmental conditions and apply no other treatments once a decision has been made to conduct Aquaflor® therapy. Complicating bacterial or parasitic diseases should be carefully documented. If necessary, these infections can be treated once Aquaflor® response (efficacy) data has been collected. However, it may take as long as several weeks after the completion of Aquaflor® therapy to determine differences between test and control groups and to complete post-treatment bacteriological evaluations.

Prior to initiating each treatment event, the Investigator must first complete a Form FFCL-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 Worksheet 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 Study Number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. The Investigator should record the assigned Study Number on Form FFCL-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 is rapidly escalating, the Investigator should contact the AADAP Office for a Study Number and permission to proceed.

X. TREATMENT GROUPS

Separately confined, untreated control groups of lobsters will not be required in all supplementary studies conducted to determine the effectiveness of Aquaflor[®] treatment. Lobsters from a group or lot will first be examined to determine if treatment with Aquaflor[®] is required. When treatment is underway or has been completed, lobsters 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 lobster mortality, although in some cases degree or severity of bacterial infestation will 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, **use of separately confined untreated control groups are strongly encouraged** whenever circumstances permit. Control groups are extremely important to not only document disease virulence and disease response to treatment, but also to validate potential adverse reactions in treated animals. Use of control groups will ensure that results of field efficacy studies provide useful information that will support an NADA. Although not required, replicate treatment groups are strongly encouraged in both treated and control groups.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which mortalities are tallied and morbidity observations recorded by individuals who are unaware which test units have been treated and which test units are serving as 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.

XI. Treatment Schedules

A. Dosage and duration:

Objective A: For the control of mortality caused by bacterial pathogens in lobsters. Aquaflor[®] medicated-feed will be fed at the rate of **10 mg of florfenicol per kg of lobster per day for 10 consecutive days.**

Objective B: For the control of mortality caused by bacterial pathogens in lobsters. Aquaflor[®] medicated-feed will be fed at the rate of **15 mg of florfenicol per kg of lobster per day for 10 consecutive days**

B. Lobster species:

Lobster stocks listed in Appendix VIa may be fed Aquaflor[®] medicated-feed in clinical field trials.

C. Feeding regime:

During the course of therapy lobsters may be fed only Aquaflor[®] medicated-feed, or a combination of medicated feed and unmedicated-feed (i.e., control 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 lobsters and the level of premix incorporated in the feed. In all situations, the daily feeding regime should be designed to optimize the rapid consumption of medicated-feed in order to ensure lobsters are treated at the intended dosage (i.e., 10 or 15 mg florfenicol per kg body weight per day).

Specify on source data sheets how lobsters were fed (e.g. % medicated-feed vs % unmedicated-feed, by hand, using automatic feeders, number of times per day feed was offered, amount of feed offered (% body weight), and whether feed was well accepted or poorly utilized.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the detection of a disease warranting Aquaflor[®] treatment. Case history records, daily morbidity and mortality records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

Morbidity and mortality data, coupled with case history and bacteriological analyses, usually indicate when Aquaflor[®] treatment is needed. **This source data must be collected for at least 10 days before treatment, during treatment, and for up to at least 21 days after the treatment period has ended.** Collection of this data is critically important in all cases. Samples of hemolymph may be collected from representative lobsters near the end of the posttreatment period and incubated/tested to determine the presence/absence of target pathogens.

2. Secondary Parameters

Secondary parameters include observations on the acceptability/consumption of medicated-feed, growth data from treated vs untreated fish, or other observations culturists believe relate directly to Aquaflor[®] therapy. Specify on source data sheets how lobsters were fed (e.g. by hand, using automatic feeders, utilizing demand feeders) and whether feed was well accepted or poorly utilized

3. Adverse Reactions

All treated lobsters should be closely observed for signs of aversion (rejection) to medicated-feed or clinical signs of drug toxicity. Any adverse reactions to treatments should be documented on source data sheets and reported immediately 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 analyses can be performed. The importance of investigator observations/comments cannot be overemphasized.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by Aquaflor® INAD 12-061 will need to complete the following forms:

- Form FFCL-W. Worksheet for Designing Individual Field Trials under INAD #12-061
- Form FFCL-1. Report on Receipt of Drug - Guide for reporting investigational new animal drug shipments for poikilothermic food animals.
- Form FFCL-2a. Chemical use log for clinical field trials using Aquaflor® medicated-feed under INAD #12-061 - Aquaflor® Premix.
- Form FFCL-2b. Chemical use log for clinical field trials using Aquaflor® medicated-feed under INAD #12-061 - Aquaflor® Medicated-Feed.
- Form FFCL-3. Diagnosis, treatment, and mortality record for clinical field trials using Aquaflor® medicated-feed under INAD #12-061.

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. Lobsters treated at a dosage of **10 mg florfenicol/kg** body weight per day for 10 consecutive days will be maintained in culture facilities for a specified **21-day withdrawal period** (from the date of last treatment) before release or harvest. Lobsters treated at a dosage of **15 mg florfenicol/kg** body weight per day for 10 consecutive days will be maintained in culture facilities for a specified **28-day withdrawal period** before release or harvest.

No withdrawal period will be required for lobsters that will not be catchable during the above-described withdrawal periods, or are illegal for harvest during those periods. No withdrawal period shall be required for dead lobsters that will be buried or rendered into non-edible products.

The Investigator must record the disposition of all treated lobsters on Form FFCL-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Aquaflor[®] medicated-feed will be used only in the manner and by the individuals specified in the Study Protocol. Any Aquaflor[®] medicated-feed remaining at the end of a study should be disposed of in a landfill or by burial. If by chance there is a bona fide need for unused drug-treated feed immediately following completion of a treatment regimen, Investigators should consult with Study Monitors to determine if unused feed is appropriate for further use. Supplemental use of unused drug-treated feed is allowed only with Study Monitor approval. The investigational drug may not be redistributed to others not specified by the protocol and may not be retained by the Investigator after completion of the study.

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

A. Drug distribution

Intervet/Schering-Plough Animal Health's Aquaflor[®] Type A Medicated Article containing 500 grams of florfenicol per kg of premix will be the only form of the drug used by feed manufacturers to formulate medicated-feed. Intervet/Schering-Plough Animal Health will provide Aquaflor[®] for use in clinical field trials to the AADAP Office for "warehousing." The AADAP Office will in turn provide Aquaflor[®] to Investigators (or feed manufacturers) only upon receipt and approval of a completed Form FFCL-W.

See Section VII.A.6. Accountability (page 5) for additional information and details.

B. Study Monitors

The Study Monitors are generally aquatic animal health professionals with experience in diagnosing and treating aquatic animal diseases. There is one Study Monitor assigned to each facility within the USFWS that is covered by the Aquaflor[®] INAD 12-061. Non-

service facilities must have a similar Study Monitor - Investigator relationship in place. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. The Study Monitors are responsible for supervision of the trials, adherence of Investigators to the Study Protocol, and inspection of the sites.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the Aquaflor[®] itself) are already available at each lobster culture facility. Diagnosis and treatment of disease is not an uncommon occurrence at most lobster culture facilities. Lobster culturists (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

Aquaflor[®] will be administered directly by the assigned Investigator (lobster culture facility manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Aquaflor[®] will be maintained in a secure location, and only the Investigator or a person under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 5) for details and Form FFCL-W, Form FFCL-1, Form FFCL-2a, Form FFCL-2b, and Form FFCL-3 for actual forms to be used in the study.

F. Recording observations

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

G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data. Upon receipt of drug, a copy of Form FFCL-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Copies of Forms FFCL-2a and FFCL-2b should be sent to the Study Monitor at the end of the calendar year, or with a corresponding Form FFCL-3. Copies of Form FFCL-3 should be sent to the Study Monitor within 10 days of completion of a study. The Study Monitor will carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor will contact the Investigator immediately to rectify the problem.

After review, Study Monitors will forward all data to the Study Director. As stated above, the complete set of raw data will be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

Form FFCL-3. Diagnosis, treatment, and mortality record is to be completed no later than 10 days after a course of therapy is completed. The purpose of this form and supplementary data is to document the results of the treatment. In addition to the data solicited by the form, attach original source data on daily mortalities occurring in all rearing units involved in the clinical field trial during the 10-day period prior to treatment, during treatment, and during the 21-day period following the completion of drug therapy.

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

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative). Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or differently than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be contacted immediately for advice. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with the quarterly data summaries, and ultimately be submitted to the Study Director.