STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUAFLOR® (florfenicol) USE AS A FEED ADDITIVE (INAD #10-697)

Sponsor:

U.S. Fish and Wildlife Service, Fish and Aquatic Conservation

_____ _____
Sponsor Signature Date Approved

Manufacturer:

Merck Animal Health
35500 W. 91st Street
Desoto, KS 66018

Facility for Coordination of Aquaflor® as a Feed Additive INAD:

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

Proposed Starting Date:	February 1, 2009
Proposed Ending Date:	December 31, 2026
Study Director:	Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility: _____

Investigator: _____

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STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUAFLOR® USE AS A FEED ADDITIVE UNDER INAD #10-697

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of feeding Aquaflor® to cultured fish to control certain bacterial diseases. INAD #10-697. **Note: No clinical field trials will be conducted under this INAD for use patterns for which Aquaflor® has already received FDA-approval (e.g., treatment of ESC in catfish, treatment of coldwater disease or furunculosis in freshwater-reared salmonids, treatment of columnaris in freshwater-reared finfish, and treatment of streptococcal septicemia in freshwater-reared warmwater finfish (NADA 141-246)).**

II. SPONSOR

Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: marilyn_j_blair@fws.gov

Manufacturer:

Merck Animal Health
35500 W. 91st Street
Desoto, KS 66018

Contact Person at Merck Animal Health:

Jackie Zimmerman
Phone: (208) 603-0336
email: jacqueline.zimmerman@merck.com

or

Merck Animal Health Customer Service
Phone: 1-800-521-5767
email: Customerservice@merck.com

Study Director: Ms. Bonnie Johnson, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9905; Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program
4050 Bridger Canyon Road, Bozeman, MT 59715;
Phone: 406-994-9911; Email: paige_maskill@fws.gov

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: February 1, 2009

Proposed Completion Date: December 31, 2026

V. BACKGROUND/PURPOSE

Aquaflor® is currently approved in the United States for treatment of ESC in catfish, treatment of coldwater disease or furunculosis in freshwater-reared salmonids, treatment of columnaris in freshwater-reared finfish, and treatment of streptococcal septicemia in freshwater-reared warmwater finfish (NADA 141-246). If your treatment is for an approved use then the INAD will not be used.

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 Merck Animal Health.

Bacterial diseases remain 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).

The efficacy of florfenicol against furunculosis in Atlantic salmon, *Salmo salar*, has been demonstrated in several studies (Samuelsen et al., 1998; Nordmo et al., 1994). Efficacy has also been demonstrated against other fish diseases, such as pseudotuberculosis in yellowtail (buri), *Seriola quinqueradiate*, (Yasunaga and Yasumoto 1988) and vibriosis in goldfish, *Carassius auratus,* and infections by *Edwardsiella tarda* in Japanese eel *Anguilla japnica* (Fukui et al. 1987). Aquaflor® is currently approved in Canada for the control of furunculosis in Atlantic salmon.

Florfenicol has great potential for treatment of infectious diseases, and because of existing data on human food safety and high potency, it could become a major drug in veterinary medicine, with special value in animal foods (Powers et al. 1990). Thus, Aquaflor® has become a strong candidate for use in aquaculture, and there is considerable interest by the aquaculture community in the U.S. to pursue approval of this drug for use in fish culture by FDA.

The objective of these field based clinical efficacy trials is to evaluate the efficacy of Aquaflor® medicated feed treatment to control mortality in a variety of fish species caused by pathogens susceptible to florfenicol. 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) enteric redmouth; and 2) bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads.

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 Aquaflor® as a feed additive to control certain bacterial diseases of fish that occur in a variety of environmental conditions, at a wide range of temperatures, and in a variety of cultured fish species. **Note: no clinical field trials will be conducted under this INAD for use patterns for which Aquaflor® has already received FDA-approval (e.g., treatment of ESC in catfish, treatment of coldwater disease or furunculosis in freshwater-reared salmonids, treatment of columnaris in freshwater-reared finfish, and treatment of streptococcal septicemia in freshwater-reared warmwater finfish (NADA 141-246)).**

2. Provide an opportunity for fish culturists to legally use Aquaflor® as a feed additive to control certain bacterial diseases of fish that occur in 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 an expanded NADA(s) for the use of Aquaflor® in various fish species.

VII. MATERIALS

A. Test and Control Articles:

1. Drug Identity

a. Active ingredient

Common Name: Florfenicol

Product Name: Aquaflor®

Chemical Description: Merck Animal Health's feed additive Aquaflor® containing 500 grams of florfenicol per kg of premix will be the only form of the drug used by fish food manufacturers to formulate treated feed, or by Investigators to top-dress feed.

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.

Molecular formula: $C_{12}H_{14}NO_4C_{12}FS$

Molecular weight: 358.20

CAS Number: 73231-34-2

Appearance and odor: White amorphous lumpy powder

b. Strength and dosage form

Drug concentration in the diet and feeding regimens will be designed to provide a daily dosage of either 10 or 15 mg of active drug per kg of fish. For treatment calculation purposes, Aquaflor® contains 50% active ingredient

c. Manufacturer, source of supply

Merck Animal Health
35500 W. 91st Street
Desoto, KS 66018

Contact Person at Merck Animal Health:

Jackie Zimmerman
Phone: (208) 603-0336
email: jacqueline.zimmerman@merck.com

or

Merck Animal Health Customer Service
Phone: 1-800-521-5767
email: Customerservice@merck.com

Note: A Veterinarian Feed Directive (VFD) is not needed when Aquaflor® is used under the INAD. Investigators will need to fill out Form FCC-W (study request) in the online database and advance the study to stage 3. AADAP will then review the study; assign a study number; then email a copy of the approved Form FCC-W to the feed mill to show use will be under an INAD. The feed mill will retain a copy of Form OTC-W for their records.

2. Verification of Drug Integrity/Strength

Merck 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.

The lot number and date of manufacture for each batch of Aquaflor® medicated feed or premix 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 FFC-1) will clearly identify the lot number and date of manufacture of all Aquaflor® shipments. If the integrity of the Aquaflor® is compromised (i.e., by spilling or contamination of the stock container) the Aquaflor® medicated feed or premix must not be used for treatment, and the event should be carefully recorded, dated, and signed in the Chemical Use Log (Form FFC-2a or FFC-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 concentrations of Aquaflor® premix to assure that target dosages of either 10 or 15 mg florfenicol/kg fish/day are achieved.

The Investigator may also prepare his/her own drug-treated feed by top-dressing feed on-hand (or specially ordered feed) with Aquaflor® premix. If the Investigator chooses this option, they are encouraged (but not required) to have a sample of the top-dressed feed assayed for florfenicol concentration by a certified, analytical testing laboratory. Results of drug-treated feed assays should be reported on Form FFC-3.

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.

Premix should be stored at temperatures and for periods of time not to exceed the limits set by Merck Animal Health.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Safety Data Sheet (SDS) 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 SDS. Safety precautions as outlined in the SDS 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 transportation emergencies telephone CHEMTREC, 800/424-9300.

5. Investigational Labeling

A copy of the label to be attached to each container of Aquaflor® medicated feed is

provided in Appendix V. Although investigational labels will be affixed to medicated feed containers by the feed manufacturer, it is the responsibility of the Investigator to ensure proper labeling of all containers of Aquaflor® medicated feed.

6. Accountability

The Online INAD Database must be used by Investigators for ALL INAD reporting. The online INAD database has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting and accountability follows established INAD Study Protocol guidelines. Unless data is entered directly into the online INAD database (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the online INAD database) investigators must archive hard copies of all raw data.

1. All facilities using Aquaflor® treated feed or Aquaflor® premix:

Immediately upon receiving an order/shipment of Aquaflor® treated feed or Aquaflor® premix, the Investigator must complete Form FFC-1 "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (located in the "Manage/View Drug Inventory" section of the investigator account). The Study Director will forward a copy of this form to the FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form FFC-1s are received by the Study Director within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of Aquaflor® treated feed or Aquaflor® premix. A Chemical Use Log (Form FFC-2a or FFC-2b) must be completed and maintained by each Investigator. Each time Aquaflor® treated feed is used, it must be recorded by the Investigator in the Results Report form in the "Amount Of Drug Used" table.

At the conclusion of field trials, all remaining Aquaflor® treated feed or Aquaflor® premix will be destroyed by following the SDS (note: unless medicated feed is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all Aquaflor® treated feed or Aquaflor® premix must be properly recorded and accounted for on the Chemical Use Log (Form FFC-2a or FFC-2b). The Study Monitor will be responsible for verifying the quantity of Aquaflor® treated feed or Aquaflor® premix remaining on hand versus the amount indicated on Form FFC-2. **Note:** Aquaflor® treated feed or Aquaflor® premix can be transferred to other facilities that are participating under INAD 10-697. Transfers must be shown in the Drug Inventory section of the database (formerly Form FFC-2a or FFC-2b).

7. Preparation Procedures

   Aquaflor® will be supplied to Investigators either as Aquaflor® treated feed or Aquaflor® premix. Neither product should be adulterated in any manner prior to use. If Investigators are using Aquaflor® premix to make their own Aquaflor® treated feed, Aquaflor® premix should be top-coated on feed. Top-coating procedures should include "finishing" with 0.5% vegetable oil.

B. Items Needed for Sample Collection, Observations, 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 bacterial culture growths microscopically.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the Aquaflor® INAD will need to complete several forms located in the online INAD database. These forms are described in Section XIII. Copies of these forms are attached to this Study Protocol and will be used as a guide only for collecting the data that will be entered into the online INAD database.

VIII. EXPERIMENTAL UNIT

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

IX. ENTRANCE CRITERIA

A. The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol for the current calendar year before Aquaflor® as a feed additive 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). However, poor planning and/or a lack of preparation will not be considered an emergency situation.

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 FFC-3. Drug discharge must be in compliance with local **NPDES** permitting requirements.

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).

Prior to initiating each treatment event, the Investigator must first complete Form FFC-W. "Worksheet for Designing Individual Field Trials" (located under the "New Study Request" tab in the investigator account) that pertains to each specific treatment event. The worksheet should be filled out and forwarded to the Study Monitor through the online INAD database. The Study Monitor will review the planned treatment (worksheet) and forward it to the Study Director at the AADAP Office. The Study Director will then review the worksheet, assign the approved treatment a Study Number, and then the online INAD database will 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 any paper forms that are being used as a guide to collect the data to enter in the online database (i.e., Form FFC-2 and FFC-3), as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach the Study Monitor with regards to Worksheet approval and the need for treatment is immediate, the Investigator should contact the AADAP Office for permission to proceed.

Note: The online INAD database, which must be used by Investigators for all INAD reporting, has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting follows established INAD Study Protocol guidelines.

E. Pathogen/disease considerations

1. Bacterial fish pathogens should be presumptively identified by procedures described in Section 1, Chapter 1 of the American Fisheries Society/Fish Health Section Blue Book "Suggested Procedures for the Detection and Identification of Certain Finfish and ShellfishPathogens, 2005 Edition. 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 2005 Edition of the "Blue Book" should be described on a separate sheet attached to Form 3 "Diagnosis and Treatment Record"**).

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 agent must be identified.

X. TREATMENT GROUPS

A. A treatment group or experimental unit may be an entire tank, pond, raceway, 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 and safety of Aquaflor® as a feed additive. Fish from a group or lot will first be examined to determine if treatment with Aquaflor® as a feed additive 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.

C. Although as stated above untreated control groups are not a required element of treatment under this INAD exemption, **it is important for all investigators to note that field trials conducted under a more stringent study protocol (i.e including requirements for non-treated controls groups, replication, blinding, dose verification, etc.) will ultimately be required in order to support a NADA for Aquaflor® as a feed additive. It is also important to note that the INAD sponsor fully expects that a limited number of facilities/investigators listed under this INAD exemption will agree to participate in such "pivotal" efficacy studies.** These studies will be initiated only after direct consultation between facilities/investigators and the sponsor. These studies will be conducted under a separate FDA-approved study protocol (i.e. not the INAD study protocol), and will also be conducted with assistance from, and under the direct supervision of, the sponsor. **If for any reason it becomes apparent to the sponsor that facilities/investigators listed under this INAD are not willing to participate in such "pivotal" studies, the sponsor will request that FDA terminate the INAD.**

XI. Treatment Schedules

A. Route of administration

Aquaflor® will be administered only as a medicated feed treatment.

B. Dosage and treatment duration

Objective A For the control of mortality caused by a variety of bacterial pathogens, in a variety of fish species, and under a variety of environmental conditions. Aquaflor® will be fed at the rate of **10 mg of florfenicol per kg of fish per day for 10 consecutive days**.

Objective B For the control of mortality caused by a variety of bacterial pathogens, in a variety of fish species, and under a variety of environmental conditions. Aquaflor® will be fed at the rate of **15 mg of florfenicol per kg of fish per day for 10 consecutive days**.

C. Dosing interval and repetition

Aquaflor® will be administered as a single treatment regimen, with no repetition of treatment.

D. Fish species

Fish stocks listed in Appendix VIa may be fed Aquaflor® treated feed in clinical field trials.

E. Feeding regimen

During the course of therapy fish may be fed only treated feed, or a combination of treated and untreated feed. The actual feeding regimen 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 some cases, feeding fish only treated feed may work best. In other cases, feeding fish treated feed first (i.e., early in the day) followed by the feeding of untreated feed may be determined to be the optimal feeding regimen. In still other cases, a small amount of untreated feed followed by a "full course" of treated feed may be utilized. However, in all cases, the daily feeding regimen should be designed to maximize consumption of the treated feed to result in consumption of the intended dosage of either 10 or 15 mg florfenicol per kg body weight.

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 minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with Aquaflor®.

However, if concomitant therapy is required in order to protect valuable fish stocks (i.e., threatened and endangered species not for human consumption) it should be fully documented and the efficacy data from the Aquaflor® treatment involved should be appropriately labeled. Contact the AADAP Office for the information that will need to be provided in the Form FFC-3 if concomitant therapy is conducted.

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 FCC-3. 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. **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 in all cases. 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.

2. Secondary Parameters

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

3. Adverse Reactions

   Any adverse reaction to treatment should be reported **immediately** to the Study Monitor, who will in turn notify the Study Director. Such responses might include changes in water quality, extremely negative responses/behavior by fish, or hazards to the applicator. Although Aquaflor® 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 for Aquaflor® medicated feed has been approved and treatments are scheduled, the Investigator at each facility covered by the INAD will need to complete the following forms:

Form FFC-W. Worksheet for Designing Individual Field Trials under Aquaflor® INAD 10-697 - located in the New Study Request tab

Form FFC-1. Report on Receipt of Drug - Guide for Reporting Investigational New

Animal Drug Shipments for Poikilothermic Food Animals – located in the Manage/View Drug Inventory tab

Form FFC-2a. Chemical Use Log for Clinical Field Trials Using Aquaflor® INAD 10-697 - Aquaflor® Premix – located in the Manage/View Drug Inventory tab and filled out in Form FFC-3 to show use

Form FFC-2b. Chemical Use Log for Clinical Field Trials Using Aquaflor® INAD 10-697 - Aquaflor® Medicated Feed – located in the Manage/View Drug Inventory tab and filled out in Form FFC-3 to show use

Form FFC-3. Results Report Form for Clinical Field Trials Using Aquaflor® INAD 10-697 – located in the Active Studies table on the home page

Copies of these forms are attached to this Study Protocol. Actual reporting is accomplished on forms located in the online INAD database.

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located in the online INAD database. All current and completed studies conducted under the investigator account will be stored and available in the online INAD database to the current study monitor, study investigator, and AADAP.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. See below for the specific withdrawal times based on fish species and treatment dose:

1. **Freshwater-reared** finfish species will be maintained at culture facilities for a specified **15-day** withdrawal period (from the date of last treatment).

2. **Saltwater-reared** finfish treated at a dose of **10 mg/kg** body weight will be maintained at culture facilities for a specified **15-day** withdrawal period (from the date of last treatment).

3. **Saltwater-reared** finfish treated at a dose of **15 mg/kg** body weight will be maintained at culture facilities for a specified **28-day** withdrawal period (from the date of last treatment).

The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form FFC-3. Also, note that the Investigator is also requested to estimate the predicted number of days/months before treated fish will be susceptible to harvest and/or human consumption on Form FFC-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Aquaflor® treated feed will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or outdated Aquaflor® treated feed remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Drug disposal information is available in the Safety Data Sheet (SDS) located in Appendix IV of this protocol. Disposition of all Aquaflor® premix or Aquaflor® treated feed medicated must be properly recorded and accounted for on the Chemical Use Log (Form FFC-2a or FFC-2b). The Study Monitor will be responsible for verifying the quantity of Aquaflor® premix or Aquaflor® treated feed medicated remaining on hand versus the amount indicated on Form FFC-2a or FFC-2b. The investigational drug may not be redistributed to others not specified by the protocol and should not be retained by the Investigator after completion of the study (note: unless Aquaflor® treated feed is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer).

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

A. Drug distribution

Merck Animal Health's feed additive Aquaflor® containing 500 grams of florfenicol per kg of premix will be the only form of the drug used by fish food manufacturers to formulate treated feed, or by Investigators to top-dress feed. Merck Animal Health will provide a limited supply of 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 FFC-W.

See Section VII.A.6. Accountability for additional information and details.

B. Study Monitors

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. A Study Monitor will be selected by each facility that is authorized to treat fish with Aquaflor® treated feed under this INAD. 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 Aquaflor® itself) are already available at each fish hatchery. Diagnosis and treatment of diseases of fish 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.).

D. Administrator of the drug

Aquaflor® will be administered directly by the assigned Investigator (fish hatchery 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 protocol Section VII.A.6. Accountability for details and the following forms will be used as guides for data collection: Form FFC-W, Form FFC-1, Form FFC-2a, Form FFC-2b, and Form FFC-3.

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 Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare summary reports 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, and must complete all required data forms (see protocol Section XIII). The Investigator should forward all completed forms to the Study Monitor for review. Study Monitors should carefully check each set of data for accuracy and completeness. If a form is incomplete or inaccurate, it should be returned to the Investigator. If a form is complete and accurate, it should be forwarded to the Study Director at the AADAP Office. **Note:** data that is entered through the online INAD database will be archived in the database. These archived forms will be available as long as the study participant accounts remain open.

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. INAD reports will be prepared and submitted to the FDA as required. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before a

field trials 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 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 out 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.** Deviations should be documented on Form FFC-3 in the *Description of Results* section and in the *Study Deviation* field.

XXI: E.O. 13891

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency policies.

Literature Cited

This information is currently not available.

Appendix I. Sponsor Contact Information for Aquaflor® INAD #10-697

Sponsor: Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9904
Fax: (406) 582-0242
Email: marilyn_j_blair@fws.gov

Sponsor Address: 4050 Bridger Canyon Road, Bozeman, MT 59715

Study Director: Ms. Bonnie Johnson
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9905
Fax: (406) 582-0242
Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9911
Fax: (406) 582-0242
Email: paige_maskill@fws.gov

Appendix II. Study Monitors for Aquaflor® INAD #10-697

Note: This information will be provided directly to CVM

Appendix IIIa. Facilities and Names of Investigators Participating under Aquaflor® INAD #10-697

Note: This information will be provided directly to CVM

Appendix IIIb. Sample of Knowledge Required for Position of Hatchery Manager (i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal, State, Tribal, and private agencies/facilities.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.

Appendix IV. Safety Data Sheet (SDS) for Aquaflor® INAD #10-697

The SDS for Aquaflor® can be found at the drug supplier's website

Florfenicol Solid Formulation_AH_US_EN.pdf (merck.com)

Appendix V. Investigational Label for Aquaflor® INAD #10-697

1. Investigational label for tests in vitro and in laboratory research animals [511.1(a)]:

   "Caution. Contains a new animal drug for investigational use only in laboratory animals or for tests in vitro. Not for use in humans."

2. Investigational label for use in clinical field trials [511.1(b)]:

   "Caution. Contains a new animal drug for use only in investigational animals in clinical field trials. Not for use in humans. Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture."

Appendix VIa. Fish Species Treated under Aquaflor® INAD #10-697

Freshwater finfish
Marine finfish

Appendix VIb. Table of Facilities and Fish Stocks Treated under Aquaflor® INAD #10-697

Note: This information will be provided directly to CVM

All data must be entered through the online INAD database:

The following forms are to be used as a guide for collecting data that will be entered into the **online INAD** d**atabase**. Any paper forms that are submitted to AADAP will be sent back to the study participants.

Form FFC-W. Worksheet for Designing Individual Field Trials under Aquaflor® INAD #10-697

INSTRUCTIONS

1. Investigator must fill out Form FFC-W for each trial conducted under this INAD before actual use of Aquaflor® treated feed.
2. Investigator should forward a copy of FFC-W to the Study Monitor for review.
3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of the Study Number.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species to be treated			
Fish species/stock to be treated			
Number of fish per unit (indicate tank, raceway or pond)			
Number of units to be treated		Number of untreated control units	
Average fish per pound		Estimated total weight of fish treated	
Intended florfenicol dosage (i.e., 10 or 15 mg florfenicol per kg fish per day)			
Projected % body weight to be fed			
Planned duration of drug treatment (days)			
Total medicated feed needed (lbs or Kg)			
Planned grams of Aquaflor® pre-mix in feed			
Anticipated treatment dates (start/end)			
Feed type (manufacturer/moist vs dry/size) for treatments and controls (identify both if different)			

STUDY DESIGN: Provide a brief description of your planned study. The description should include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by; _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

_____ Estimated time (days, months) from last treatment day to first possible harvest for human consumption

Check applicable box(es):

☐ 10 or 15 mg florfenicol per kg BW per day for 10 days; 15-day withdrawal period for freshwater-reared finfish..

☐ 10 mg florfenicol per kg BW per day for 10 days; 15-day withdrawal period for saltwater-reared finfish.

☐ 15 mg florfenicol per kg BW per day for 10 days; 28-day withdrawal period for saltwater-reared finfish.

☐ Investigator or alternate shall initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section XV. of the Study Protocol.

WORKER SAFETY CONSIDERATIONS:

_____ Initial here to indicate that all personnel handling drug have read Safety Data Sheet for Aquaflor® and are aware of SAFETY precautions to be taken when handling medicated feed.

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

FORM FFC-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

1. Investigator must fill out Form FFC-1 **immediately** upon receipt of florfenicol-medicated feed.
2. Investigator should forward a copy of Form FFC-1 to the Study Director at the AADAP Office.

The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted in triplicate:

Name of Drug	**Aquaflor®**	INAD Number	**10-697**
Proposed Use of Drug	Treatment of certain bacterial diseases that occur in a variety of fish species		
Date of CVM Authorization Letter	September 21, 2016		
Date of Drug Receipt		**Amount of Drug Received**	
Drug Lot Number		**Trial Number**	
Name of Investigator			
Address of Investigator			
Location of Trial			
Pivotal Study	**Yes**	Non-pivotal Study (yes/no)	----
Approximate Number of Treated Animals		**Approximate Number of Control Animals**	
Number of Animals Used Previously[1]			
Study Protocol Number	10-697		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	15 mg florfenicol/kg fish per day for 10 consecutive days		
Methods(s) of Administration	Medicated-feed		
Withdrawal Period	15 days for freshwater species; 15 days for saltwater species treated at 10 mg/kg bw; 28 days for saltwater species treated at 15 mg/kg bw.		

[1] To be filled out by the NIO

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____

Date Reviewed: _____ **Sponsor:** _____

Form FFC-2a. Chemical Use Log for Clinical Field Trials Using Aquaflor® as a Feed Additive Under INAD #10-697 - Aquaflor® Premix

INSTRUCTIONS

1. Initiate Form 2 immediately upon receipt of Aquaflor® premix.
2. Each lot number of Aquaflor® premix may be used for multiple treatment regimes.

Qty of on Hand
From Previous Page _____ Facility _____ Reporting individual_____

Aquaflor® Premix Lot Number	Date Received	Amount Received (ml)	Date Used	Study Number	Amount Aquaflor® Premix used for treatment (g)	Aquaflor® Premix Shipped[1] (g)	Aquaflor® Premix Disposal[2] (g)	Aquaflor® Premix on hand (g)	Inventory by (initials)

[1] Unused Aquaflor® Premix that is shipped to another facility participating in Aquaflor® INAD #10-697 (**Note**: Aquaflor® Premix can only be shipped to another facility with prior authorization by the AADAP Office).

[2] Unused Aquaflor® Premix that is disposed of by burial or in a landfill.

Investigator: _____ **Study Monitor:** _____
Signature and Date Signature and Date

Form FFC-2b. Chemical Use Log for Clinical Field Trials Using Aquaflor® as a Feed Additive Under INAD #10-697 - Aquaflor® Medicated Feed

INSTRUCTIONS

1. Initiate Form 2 immediately upon receipt of Aquaflor® treated feed.
2. Each lot number of Aquaflor® treated feed should be used for a single treatment regime.

Qty of on Hand
From Previous Page _____ Facility _____ Reporting individual_____

Aquaflor® Treated Feed Lot Number and % Premix	Date Received	Amount Received (lbs)	Date Used	Study Number	Aquaflor® Treated Feed Used for Teatment (lbs)	Aquaflor® Treated Feed Shipped[1] (lbs)	Aquaflor® Treated Feed Disposal[2] (lbs)	Aquaflor® Treated Feed On-hand (lbs)	Inventory by (initials)

[1] Unused Aquaflor® Premix that is shipped to another facility participating in Aquaflor® INAD #10-697 (**Note**: Aquaflor® treated feed can only be shipped to another facility with prior authorization by the AADAP Office).

[2] Unused Aquaflor® treated feed that is disposed of by burial or in a landfill.

Investigator: _____ **Study Monitor:** _____
Signature and Date Signature and Date

Form FFC-3. Results Report Form for Clinical Field Trials Using Aquaflor® as Feed Additive under INAD #10-697

INSTRUCTIONS

1. Investigator must fill out Form FFC-3 no later than 30 days after completion of treatment. Attach lab reports and other information.
2. If Aquaflor® was not used under the assigned Study Number, contact the Study Director at the AADAP Office on how to close-out the study.
3. Investigator should forward a copy of Form FFC-3 to the Study Monitor. Within 10 days of receipt, the Study Monitor should forward a copy to the Study Director at the AADAP Office.

SITE INFORMATION

Facility	
Reporting Individual	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species treated		Fish disease treated	
Average fish/pound		Average fish length	
Number of fish per experimental unit (indicate tank, raceway, or pond)			
Number of treated units		Number of control units	
Total weight of fish treated (lbs or kg)		Feed rate (% BW/day)	
Treatment duration	10 days	Total medicated feed fed (lbs or kg)	
Aquaflor® lot number		Florfenicol dosage (i.e., 10 or 15 mg per kg fish body weight)	
Aquaflor® premix used to prepare medicated feed (g)			
Feed type (manufacturer/moist vs dry/size)			
Feeding method (hand, auto, demand)			
Preparation of Aquaflor® treated feed (top-dressed at facility or prepared by feed manufacturer)			
Date treatment started		Date treatment ended	

WATER QUALITY PARAMETERS

Ave pre-treatment temp (°F)		Dissolved Oxygen (mg/L)	
Ave treatment temp (°F)		pH	
Ave post-treatment temp (°F)		Hardness - $CaCO_3$ (mg/L	

Form FFC-3. Daily Mortality Record

INSTRUCTIONS

1. Investigator should fill out the Daily Mortality Record as completely as possible.
2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is Treated or Control, and the number of fish in each rearing unit.
3. Water temperature and individual tank mortality should be recorded on a daily basis.
4. **Even if mortality is zero an entry is still needed for that day.**

FACILITY										
	Rearing Unit ID									
	Treated or Control									
	Number of Fish									
	Day	**Date**	**Water Temp (F°)**	**Mortality**	**Mortality**	**Mortali-ty**	**Mortality**	**Mortality**	**Mortality**	**Daily Observer Initials**
Pre-Treatment Period	**1**									
	2									
	3									
	4									
	5									
Treatment Period	**1**									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									
Post:Treatment Period	**1**									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									

STUDY NUMBER _____

RESULTS: Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Describe general fish behavior, including feeding behavior. Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol?

Pathology Report: Attach pathology report to this form. Report should include: 1) a description of how the pathogen(s) was identified; 2) disease identification records that confirm the presence of the pathogen; and 3) the name and title of the individual performing the diagnosis.

Pathology Report included: ☐ pre-treatment ☐ post-treatement

TOXICITY OBSERVATIONS: Report any apparent drug toxicity including a description of unusual fish behavior.

OBSERVED WITHDRAWAL PERIOD:

☐ 15 day withdrawal period for freshwater species.

☐ 15 day withdrawal period for saltwater species treated at 10 mg/kg bw.

☐ 28 day withdrawal period for saltwater species treated at 15 mg/kg bw.

Estimated number of days between last treatment and first availability of fish for human consumption (ensure this time period meets the withdrawal period). _____

DISPOSITION OF UNUSED OR SPOILED AQUAFLOR® TREATED FEED:

☐ Use and disposition of all Aquaflor® treated feed followed Study Protocol guidelines and has been clearly identified on Form FFC-2b (Investigator should initial)

☐ **NEGATIVE REPORT:** Aquaflor® treated feed was not used at this facility under this Study Number during the reporting period. The study will be closed out in the online INAD database.

Date Prepared: _____ **Investigator:** _____

Date Reviewed: _____ **Study Monitor:** _____