

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

Sponsor:

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

Sponsor Signature

Date Approved

Manufacturer:

Intervet/Schering-Plough Animal Health
1095 Morris Avenue
Union, NJ 07083-1982

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: January 31, 2012

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 A SUPPLEMENTAL AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUAFLO[®]R 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 (NADA 141-246), and treatment of columnaris in catfish (NADA 141-259)].**

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
1095 Morris Avenue
Union, NJ 07083-1982

Contact person: Dr. Richard Endris, Research Program Manager
Telephone: (908) 473-3133; Fax: (908) 473-3654

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: February 1, 2009

Proposed Completion Date: January 31, 2012

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 Schering-Plough Animal Health. For additional information on florfenicol and Aquaflor[®] see Addendum II.

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 quinqueradiata*, (Yasunaga and Yasumoto 1988) and vibriosis in goldfish, *Carassius auratus*, and infections by *Edwardsiella tarda* in Japanese eel *Anguilla japonica* (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) systemic columnaris; 2) furunculosis, 3) enteric redmouth; and 4) 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 and treatment of coldwater disease or furunculosis in freshwater-reared salmonids (NADA 141-246), and treatment of columnaris in catfish (NADA 141-259)].**
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.

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 fish species (i.e., **10 mg of florfenicol per kg of fish per day for 10 consecutive days**) is efficacious when fed as a feed additive for the control of mortality caused by 1) these same bacterial pathogens in additional species, and 2) other bacterial pathogens (including enteric redmouth, bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads, and other gram negative systemic bacteria) in a broad variety of fish species when cultured under a variety of rearing or environmental conditions. Salmonid fish species treated in this manner may be released for immediate harvest after a 21-day withdrawal period (from the date of last treatment). Non-salmonid fish species treated in this manner may be released for immediate harvest after a 28-day withdrawal period. No withdrawal period will be required for fish 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 of fish per day for 10 consecutive days** is efficacious (or in some cases possibly more efficacious than treatment at a dosage of 10 mg of active drug per kg of fish per day for 10 consecutive days) when fed as a feed additive to control mortality caused by a variety of bacterial pathogens in a variety of fish species cultured under a variety of environmental conditions. Salmonid fish species treated in this manner may be released for immediate harvest after a 21-day withdrawal period (from the date of last treatment). Non-salmonid fish species treated in this manner may be released for immediate harvest after a 28-day withdrawal period. No withdrawal period will be required for fish 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

Schering-Plough 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.

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 active drug per kg of fish.

g. Manufacturer, source of supply

Intervet/Schering-Plough Animal Health
1095 Morris Avenue
Union, NJ 07083-1982

Contact person: Dr. Richard Endris, Research Program Manager
Telephone: (908) 473-3133
Fax: (908) 473-3654

h. Additional information

See Addendum II.

2. Verification of Drug Integrity/Strength

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 FFC-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 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 Intervet/Schering-Plough Animal Health. Inventories of both treated feed and premix on-hand must be recorded on either Form FFC-2a or Form FFC-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 transportation emergencies telephone CHEMTREC, 800/424-9300.

5. Investigational labeling

Copies of the labels to be attached to each bag of Aquaflor[®] treated feed are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all bags of treated feed.

6. Accountability

1. USFWS Facilities and Non-USFWS Facilities

Immediately upon receiving an order/shipment of Aquaflor[®] treated feed or Aquaflor[®] premix, the Investigator must complete Form FFC-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 FFC-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 FFC-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form FFC-1s are received by the Study Director in a timely manner.

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

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.

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

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 Shellfish Pathogens, 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").**

Other entrance criteria for the use of Aquaflor[®] as a feed additive 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 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.
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 the fish cultural 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 FFC-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 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 FFC-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 fish will not be required in all supplementary studies conducted to determine the effectiveness of Aquaflor[®] treatment. Fish 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, 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 will also be quantitated.

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 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.

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 Aquaflor[®] treated 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. Aquaflor[®] 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 Aquaflor[®] 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. replication, randomization, blinding, etc.). Such situations are suitable for the conduct of pivotal, carefully designed and controlled studies following the Aquaflor[®] pivotal study protocol FLOR-99-EFF (available from the AADAP Office). Fish should be treated in place whenever possible without changing the circumstances bringing on the disease. These facilities will be given top priority for the availability of treated feed, assistance from monitors and AADAP personnel, and diagnostic support from fish health biologists.

XI. Treatment Schedules

A. Dosage and 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.**

B. Fish species:

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

C. 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 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 regime. 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 regime 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.

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 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

All treated fish should be closely observed for signs of aversion (rejection) to treated 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 the Aquaflor® INAD 10-697 will need to complete the following forms:

- Form FFC-W. Worksheet for Designing Individual Field Trials under INAD #10-697
- Form FFC-1. Report on Receipt of Drug - Guide for reporting investigational new animal drug shipments for poikilothermic food animals.
- Form FFC-2a. Chemical use log for clinical field trials using Aquaflor® as a feed additive under INAD #10-697 - Aquaflor® Premix.
- Form FFC-2b. Chemical use log for clinical field trials using Aquaflor® as a feed additive under INAD #10-697 - Aquaflor® Medicated Feed.
- Form FFC-3. Diagnosis, treatment, and mortality record for clinical field trials using Aquaflor® as a feed additive under INAD #10-697.

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. Salmonid fish species will be maintained at culture facilities for a specified 21-day withdrawal period (from the date of last treatment). Non-salmonid fish species will be maintained at culture facilities for a specified 28-day withdrawal period.

No withdrawal period will be required for fish 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 fish that will be buried or rendered into non-edible products.

The Investigator must record the disposition of all treated fish on Form 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. Any Aquaflor® treated 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 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. 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 FFC-W.

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

B. Study Monitors

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. There is one Study Monitor assigned to each facility within the USFWS that is covered by the Aquaflor® INAD 10-697. 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 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., page 6).

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 Section VII.A.6. Accountability (page 5) for details and Form FFC-W, Form FFC-1, Form FFC-2a, Form FFC-2b, and Form FFC-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 FFC-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Copies of Forms FFC-2a and FFC-2b should be sent to the Study Monitor at the end of the calendar year, or with a corresponding Form FFC-3. Copies of Form FFC-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 FFC-3 Report on Efficacy of Treatments 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.

Addendum 1

Additional requirements and guidelines for the use of Aquaflor® under INAD 10-697 at a dosage of 15 mg florfenicol/kg body weight/day for 10 consecutive days to control mortality caused by bacterial coldwater disease (CWD; causative agent *Flavobacterium psychrophilum*) in freshwater-reared salmonids

1. Entrance Criteria:

Prior to initiation of treatment, each facility must provide written documentation to the AADAP Office that previous treatment of specific fish stocks with Aquaflor® at a dosage of 10 mg florfenicol/kg body weight/day for 10 consecutive days to control mortality caused by CWD has been found to be non-efficacious for long-term disease control. Such documentation should be provided to the AADAP Office in the form of a letter that is signed and dated by an authorized agency/facility representative.

Additionally, a licensed veterinarian must verify the need for all treatments at the 15 mg florfenicol/kg dose. Verification of the need for treatment should be documented under "Study Design" on page 2 of Form FFC-W Worksheet for Designing Field Trials.

2. Diagnostics, Disease Confirmation, and Pathogen Identification:

Although treatment with Aquaflor® may be initiated based on observation of clinical signs of CWD, increased mortality, and station history (all of which should be factored in to the verification of treatment need as described above), isolation and identification of the etiological agent (*F. psychrophilum*) must be documented. Presumptive and confirmatory diagnosis should be completed following procedures outlined in the 2010 Edition of the American Fisheries Society's Fish Health Section Blue Book (see Chapter 1.4 Coldwater Disease, pages 1- 11), and results should be attached to Form FFC-3. Diagnosis, Treatment, and Mortality Record.

3. Susceptibility Testing, Minimum Inhibitory Concentration (MIC) Determination, and Archiving of Isolates

Although not required, all Investigators are strongly encouraged to 1) conduct florfenicol disk diffusion susceptibility testing on isolates, 2) determine minimum florfenicol inhibitory concentration utilizing Sensititre Plates, and 3) lyophilize and archive all *F. psychrophilum* isolates. Florfenicol susceptibility disks and Sensititre Plates are available at no cost from the AADAP Office. Additionally, isolate cultures may be shipped to the AADAP Office and AADAP staff will assume responsibility for isolate susceptibility testing, MIC determination, and archiving.