A RESEARCH PROTOCOL TO DETERMINE:

The Efficacy of Florfenicol Medicated Feed to Control Mortality Caused by Pathogens Susceptible to Florfenicol in a Variety of Fish Species.

Protocol FLOR-01-EFF

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Testing Facilities: Federal, State and Private Facilities throughout the United States

Reviewed and Approved by:

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1. INTRODUCTION:

1.1 Objective:

1. To evaluate the efficacy of florfenicol medicated feed fed at a rate of 10 mg of active drug per kg of fish per day for 10 days to control mortality caused by pathogens susceptible to florfenicol in a variety of fish species.

2. $H_o : u_{\text{treated}} = u_{\text{untreated}}$

   No significant difference exists in mortality caused by pathogens susceptible to florfenicol in groups of test fish treated with florfenicol medicated feed fed at a rate of 10 mg of active drug per kg of fish per day for 10 days, and groups of test fish fed unmedicated feed.

3. $H_a : u_{\text{treated}} < u_{\text{untreated}}$

   Mortality associated with pathogens susceptible to florfenicol will be lower among groups of test fish treated with florfenicol medicated feed fed at a rate of 10 mg of active drug per kg of fish per day for 10 days than among groups of test fish fed unmedicated feed.

4. Study Director, Investigator, Study Monitor and study personnel involved in the conduct of the study will be thoroughly familiar with the Study Protocol for the Efficacy of Florfenicol Medicated Feed to Control Mortality Associated with Pathogens Susceptible to Florfenicol in a Variety of Fish Species - Protocol FLOR-01-EFF; the supplemental USFWS Compassionate Florfenicol INAD exemption protocol #10-697, and the Conduct of Clinical Investigations: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Studies (FDA document dated Oct. 1992 or later - see Attachment II).

1.2 Background and Justification:

Florfenicol is a potent, broad spectrum antibacterial agent with bacteriostatic properties (Horsberg et al 1996). It is a flourinated analogue of thiamphenicol and is also similar in structure to chloramphenicol, both of which have also been used as broad spectrum veterinary antibiotics (Nagata and Oka 1996).

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 Japaness eel *Anguilla japnica* (Fukui et al. 1987).

Florfenicol has great potential for treatment of infectious diseases and because of its 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, it has become a strong candidate for use in aquaculture, and there is considerable interest to get this drug approved for use in fish culture by FDA.

The objective of these field based clinical efficacy trials is to evaluate the efficacy of florfenicol-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 diagnosed with a variety of fish pathogens, and showing signs or symptoms of bacterial disease(s), such as coldwater disease, columnaris, furunculosis, enteric redmouth, bacterial hemorrhagic septicemia caused by Aeromonads and Pseudomonads and other gram negative systemic bacteria.

Efficacy studies will be conducted at facilities after the following have been considered: 1) history of predictable, recurring outbreaks of fish diseases of interest at the potential study sites; 2) each site has space at the hatchery to dedicate to conducting studies; 3) each site has additional resources available to conduct studies (i.e. - test units, test animals, staff to monitor study and collect data); 4) and each has demonstrated commitment to adhere to Protocols and Guidelines. Studies will be conducted under the Pivotal Study Protocol FLOR-01-EFF and are intended to provide the FDA/Center for Veterinary Medicine (CVM) with pivotal clinical field efficacy data.

2. INVESTIGATIONAL DRUG AND CONTROL:

2.1 Test Substances:

2.1.1 Trade name:
Florfenicol; Aquaflor® (each kg of Aquaflor® premix contains 500 g (50%) of florfenicol in a palatable base)

2.1.2 Chemical name - active component(s):
D-(threo)-1-(p-mthylsulfonylphenyl)-2-dichloroacetamide-3-fluoro-1-propanol.

2.1.3a Molecular formula
$C_{12}\ H_{14}\ N\ O_4\ C_{12}\ F\ S$

2.1.3b Molecular weight
358.20

2.1.4 Appearance and odor:
White amorphous powder

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

2.1.6 Dosage Form
Aquaflor® will be top-coated on commercially prepared feed, or mixed in unmedicated feed before pelleted.

2.1.7 Dose(s) to be tested
10 mg florfenicol per kg body weight per day

2.1.8 Manufacturing site
Schering-Plough Animal Health
Division of Schering Canada Inc.
3535 Trans-Canada
Pointe Claire, Quebec H9R 1B4
514-426-7300
514-695-7641 fax

2.1.9 Lot Number
UK-1-BGCA-01. If lot number of the Aquaflor® premix differs from the lot number identified here, the different lot number will be included in the final report.

2.1.10 Packaging

Aquaflor® is packaged in individual 2 kg sealed laminated foil pouches.

2.1.11 Storage conditions

Aquaflor® will be stored in a dry place between 2 - 30° C. Premix will be used within 12 months of opening pouch. Date pouch was opened will be recorded on the pouch.

2.1.12 Drug storage during study.

Aquaflor® premix will be stored as above. Florfenicol medicated feed will be stored according to feed storage instructions, which typically recommend storing in a cool, dry place. Medicated feed will not be used more than 6 months after manufacture of the feed. Feed manufacture data will be included in the final study report.

2.1.13 Drug handling procedures:

The test article will be handled in accordance with precautions described in the Material Safety Data Sheet (MSDS). A copy of the MSDS will be available to all personnel in the study.

Florfenicol has been shown to pose a low level of inhalation toxicity, may produce skin and or eye irritation in sensitive individuals, and is classified as slightly hazardous via oral ingestion.

2.1.14 Verification of drug integrity/strength:

Feed will be assayed for drug strength by scientists at the United States Geological Services Upper Midwest Environmental Sciences Center (UMESC), LaCrosse WI (see Section 6.5.1.1.4 for address and telephone numbers).

2.1.15 Investigational labeling:

Copies of investigational labels will be attached to each container (i.e., pouch) of florfenicol medicated feed. It will be the responsibility of the Study Director and Investigator to ensure proper labeling of all containers of florfenicol medicated feed.

2.1.16 Accountability:

The FDA will be notified when florfenicol (Aquaflor®) is ordered from the manufacturer. A drug use log will document use of Aquaflor® premix.

2.1.17 Material Safety Data Sheet (MSDS)

A complete MSDS is included in Appendix I.

2.2 Controls:

Controls will be test fish that are fed unmedicated feed. Control fish and fish in treated tanks will be fed similar amounts, size, and type of feed. Control and treated fish will be fed at the same time and at the same feeding frequency (e.g., 4 times per day).

3. STUDY SCHEDULES:

3.1 Proposed date(s) of initiation:

Proposed dates of initiation will be study site specific. Studies will be conducted at: (1) U. S. Fish and Wildlife Service (Service) facilities; (2) State hatcheries; (3) private hatcheries or research labs; and or (4) tribal hatcheries. Studies will be initiated when there is a disease outbreak and daily mortality is sufficient to adequately address the study objective. Ideally, the Study Director will be available to assist initiating the medicated-feed treatment portion of the study. However, if the Study Director cannot be on-site, then the Investigator on station will initiate the study with telephone support from the Study Director or his designee. Date of study initiation and significant events in each study will be summarized in the final report.

3.2 Schedule of events:

3.2.1 Transfer of test animals:

Fish will be transferred into test units no less than 1 d prior to initiation of treatment. Allocation of fish will be done in a manner to minimize bias among tanks with respect to the level of fish disease. All information pertaining to transfer of fish to test units, including randomization procedures used to determine allocation of fish to test tanks, dates of fish transfer to test tanks, and study initiation, will be described in the methods section of the final report.

3.2.2 Initiation of treatment:

Treatment will begin no sooner than 1 d after fish have been transferred to test units.

3.2.3 Treatment period duration:

The treatment period will begin the day treatment is initiated (day 1) and will last 10 days.

3.2.4 Post-treatment period duration:

Duration of the post-treatment period will be at least 14 d, but may be as long as 30 days.

3.2.5 Data analysis and report writing:

Data collection, analysis and preparation of final report will require a maximum of 12 months after the study termination date to complete. Within 12 months of completion of post-treatment period, a final report will be submitted to the Food and Drug Administration/Center for Veterinary Medicine.

3.3 Proposed date(s) of completion:

The data collection portion of each study should be completed within 40 d of the initiation of treatment. Final date of completion of individual studies will be no later than 12 months after completion of the study. The proposed date for completion of all studies will be June, 2004.

4. STUDY DESIGN:

4.1 Treatment groups:

Studies will typically consist of a single treated group and a single non-treated (control) group. All fish in a particular study will consist of fish from the same fish lot. Treated groups will be treated at 10 mg florfenicol per kg fish body weight per day for 10 days. Control groups will receive non-medicated feed at the same feed rate as the treated fish. There will be at least three replicates per treatment group.

4.2 Experimental design:

The experimental design typically used will be completely randomized. If blocking factors are suspected, then a block randomized design will be used.

4.3 Blocking factor(s):

It is anticipated that no blocking factor(s) will be used in the study design. All rearing units will be plumbed with the same water supply in a confined area in a hatchery building or bank of raceways. All units will be exposed to the same ambient temperature and photoperiod, and fed the same diet. Water chemistry conditions, such as temperature, dissolved oxygen, pH, and hardness will be consistent among all rearing units. All rearing units will experience similar foot traffic and light conditions. However, if blocking is deemed appropriate, a description of the blocking factors will be included in the final report.

4.4 Randomization procedures:

4.4.1 Allocation of animals to test units:

Animals will be randomly placed in test units in such a manner as to minimize bias. Each test tank will be uniquely numbered. At each stage of the allocation process, a given test tank will receive no more than approximately 10 - 50% of the total number of fish to be ultimately transferred to each test tank. If possible, fish will be allocated to test tanks according to SOP No. MISC 205. Otherwise, an acceptable randomization technique will be used to determine which test unit will receive the first group of fish, which will receive the second, and so on until fish have been transferred into all test tanks. Following this procedure, each test tank will receive subsequent groups of fish following the same order as described above until each test tank holds a full complement of test fish.

4.4.2 Allocation of treatment groups to experimental units:

After all fish have been transferred to test units, the treatment condition (i.e., treated or untreated) will be randomly assigned to each test tank according to SOP No. MISC 206.

4.5 Configuration of experimental units:

Configuration of experimental test tanks could be different at each facility. Test tanks will be set up according to recommendations from the on-site Investigator or their designee on how to best accommodate their inclusion at the study site. All test tanks will be plumbed in the same manner. Each test tank will receive water from the same source and hold equal numbers of test fish. The configuration of experimental test tanks will be described in the methods section of the final report.

5. STUDY PROCEDURES:

5.1 Test Animals:

5.1.1 Description:

5.1.1.1 Age/Size:

Fish used in the studies will be characterized as early life stage, (i.e., fry, fingerling, or juveniles between 1 - 10 inches in length; see Table 5.1.2). Fish species and size (total length and weight) will be described in the final study report.

5.1.1.2 Sex:
Sex of fish will not be determined or considered; however, it will be assumed that males and females will be present in roughly equal proportions. It is also assumed that sex will not be a factor in treatment efficacy.

5.1.1.3 Breed/Class:
Fish species will be confirmed by the Hatchery Manager.

5.1.1.4 Initial body weight:
Initial body weight and length of test fish used in each study is not known at this time. Initial body weight will be determined by sample counts or by measuring the mean length of a group of fish and determining weight using length-weight relationship tables (Piper et al. 1982) on or before the first day of the study. This information will be documented and described in the final study report.

5.1.1.5 Physiological state:
The physiological state of test fish will not be determined nor considered; however, fish will be "sick", and at least some of the test fish will be showing signs and symptoms of the disease.

5.1.1.6 History of test animals:
Test fish will most likely be from eggs incubated at the hatchery where the study will take place, or brought into a facility at an early life-stage. Test fish will typically be reared on site under standard hatchery conditions as described by Piper et al. (1981). A brief description will be included in the final report detailing information such as egg source, egg incubation procedures, management practices, and environmental conditions under which test fish were reared prior to study (e.g., type and size of fish, flow and density index values, water turnover rates, rearing temperature, pH, hardness, dissolved oxygen), and any therapeutic chemical treatment administered prior to the study. If such treatment has been deemed necessary, the reason why treatment was necessary will be included in the final study report.

5.1.2 Number of test animals:
A sufficient number of fish will be held in each test tank to approximate near-production loading. Density index (DI) and flow index (FI) values, which are indicators of whether or not hatchery-

reared fish are being maintained within the carrying capacity of a given rearing unit, will not exceed that recommended by Piper et al. (1982).

5.1.2.1 Flow index:

A flow index (FI) value relates total weight of fish per tank to the tank's water inflow and mean length of the fish. Note that FI ranges will differ for different fish species at a given facility. Flow index is calculated by the following formula:

$$FI = \frac{(\text{total number of fish})*(\text{mean weight of fish (lbs)})}{(\text{mean length of fish (in.)})*(\text{water flow rate (gal./minute)})}$$

As the number of fish decreases in a particular test unit as a result of mortality, the FI will also decrease. As the study progresses, no steps will be taken to maintain FI at the level set at the start of the study. Although fish will be fed during the course of study, no appreciable growth or weight gain is expected. If necessary, water flow to test tanks will be readjusted during the study to maintain initial water flow.

5.1.2.2 Density index:

A density index (DI) value relates total weight of fish per tank to the tank's rearing volume and mean length of fish. Note that DI ranges will differ for different fish species at a given facility. Density index is calculated by the formula:

$$DI = \frac{(\text{total number of fish})*(\text{mean weight of fish (lbs)})}{(\text{mean length of fish (inches)})*(\text{size of test unit (in cubic feet)})}$$

As the number of fish decreases in a particular test unit as a result of mortality, the DI will also decrease. As the study progresses, no steps will be taken to lower the volume of water in the test unit to maintain the DI at the level set at the start of the study. Although fish will be fed during the course of study, no appreciable growth or weight gain is expected. Note that FI and DI values are commonly used to determine loading rates for salmonids. If these methods are not appropriate for other fish species (i.e., shovelnose sturgeon), then an accepted method to determine loading rates will be used and cited in the final study report.

5.1.3 Source of animals:

Source of test animals will be documented in an appropriate manner. At this time, the sources of test fish to be used in the various study sites are not known.

5.1.4 Identification method if not client-owned companion animals:
Test animals will not possess any artificial or man-made identification. Precautions will be taken to ensure test fish from one test tank do not escape or mix with test fish from another test tank by either covering test tanks with material to prevent escapement, or by placing test tanks in locations such that fish can not jump from one test tank to another.

5.2 Inclusion criteria:

The entrance criteria for inclusion in the study will include the following: 1) study fish will have been diagnosed with pathogens susceptible to florfenicol, and fish-pathogen related mortality will be adequate to sufficiently address the study objective; 2) fish are known not to have any secondary disease pathogen; 3) the on-site Investigator or his designee is on site to allocate test fish to test tanks and initiate start of treatment; and 4) enough test units and test animals are available to conduct studies in triplicate under near production-like conditions. The Study Director, Study Monitor, Investigators, and other personnel involved in each study shall be thoroughly familiar with protocols and guidelines listed in Section 1.1 paragraph 4, and will be committed to following through with the study until completion.

Diagnosis of pathogens susceptible to florfenicol will be confirmed by the Study Monitor using appropriate and accepted procedures. These procedures will be described in the final study report.

5.2.1 Ability of investigator to fulfill all the requirements of the protocol:

Investigator(s) will be fully capable of ensuring all requirements of the protocol are fulfilled. The Study Director will working in cooperation with the Investigator(s) to ensure that the protocol is followed. Study sites were selected, in part, because Investigator(s) have experience using medicated feed in accordance with compassionate INAD exemption protocols to treat for systemic diseases. The Investigator(s) shall be thoroughly familiar with the Pivotal Study Protocol for Efficacy of Florfenicol Medicated Feed Treatment for the Control of Mortality caused by Pathogens susceptible to florfenicol - Study Protocol FLOR-01-EFF; and with Conduct of Clinical Investigations: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Studies (dated Oct. 1992 or later - see Attachment II).

5.2.2 Level of disease:

Ideally, there should be increased morbidity or mortality rates among fish with disease signs typical of systemic bacterial infections. Typical disease signs should be detectable in at least a few fish and the causative

bacterial agent identified in moribund fish. However, the level of the disease should be relatively low, or in the early stages of development, to obtain control by orally administering florfenicol medicated-feed. If the level of the disease is too advanced, fish may have such deep-seated chronic infections that near-total mortality is imminent. Therefore, prompt diagnosis and treatment is imperative.

5.3 Exclusion criteria:

A test unit will be excluded from the study if a "fatal" event occurs, such as: (1) multiple disease infections among test fish; (2) water flow interruption for a period of time that unduly stresses test fish; (3) a standpipe is left out resulting in dewatering of the test tank for a period of time that unduly stresses test fish; or (4) untreated test fish experience "spontaneous recovery" and mortality returns to baseline levels. Fish used in section 5.2 to determine disease uniformity will be evaluated using a standard fish health diagnostic scheme to determine if other infectious pathogens are present in sufficient numbers to adversely affect the outcome of the study (see Appendix II). If it is discovered that mortality among diseased fish may not be attributable to bacteria susceptible to florfenicol, then steps for exclusion will be taken. If exclusion of any test unit is deemed necessary, it will be documented and described in the final study report.

5.4 Acclimation of test animals:

5.4.1 Duration:

Test conditions, including the acclimation period, will be nearly identical to conditions fish were reared in when: (1) fish initially became infected with the bacterial pathogen; (2) fish began to show disease symptoms; and (3) fish-pathogen mortality increased. The 1 d time period between when fish are transferred to test units and when the medicated-feed treatment begins will be termed the acclimation period. If a longer acclimation period is used, the duration and reasons for using a longer acclimation period will be described in the final study report.

5.4.2 Medication and/or vaccination during acclimation period:

During the acclimation period, no medication and/or vaccinations will be administered to the test fish.

5.4.3 Baseline data collected prior to initiating study:

Daily mortality in the rearing units holding the reference or study population will be collected prior to allocating fish to test tanks or initiating the treatment phase of the study. A sample of fish from the reference population will be examined for fish pathogens before initiating the study to confirm that mortality is related to a specific fish pathogen.

5.5 Blinding of study:

5.5.1 Extent of blinding:

At least one study participant will be non-blinded, and know the treatment condition of each test tank. Non-blinded study participant(s) will not be involved in the day-to-day data collection during the course of the study. Blinded study participants will collect data critical to addressing the study objective, such as mortality, as well as measure daily water quality parameters and conduct routine fish care and feeding. Non-blinded study participants will be responsible for weighing out medicated and unmedicated feed, and coordinating fish feeding with blinded study participants. Some tasks, such as allocating fish to test tanks, measuring water hardness, alkalinity, pH, and collecting and shipping feed samples to be assayed for drug concentration, may be done by either blinded or non-blinded study participants.

5.5.2 Blinding method(s) and procedure(s):

A single blinding method will be used.

5.5.3 List of personnel with access to treatment codes and rationale:

Study personnel with access to treatment codes is not known at this time, but will be determined before the start of each study. The Study Director and on-site Investigator will agree which study personnel will be designated to have access to the treatment codes. At least one study participant with access to treatment codes will possess sufficient scientific background to assist in the study, but will not play a role in the day-to-day activities of the study.

5.6 Drug Administration:

5.6.1 Dosing regime:

The dosing regime for all studies will be 10 mg florfenicol/kg fish/day. Fish will be fed daily for 10 consecutive days.

5.6.2 Route of administration:

All medicated feed will be administered orally. Fish will be fed a commercial fish feed that is either top-coated with florfenicol premix, or in which florfenicol premix has been incorporated.

5.6.3 Investigational withdrawal period:
The investigational withdrawal period will be 21 days (see Appendix III).

5.6.4 Proposed withdrawal period:
The proposes investigational withdrawal period will be 21 days.

5.7 Removal of subjects from study:

5.7.1 Criteria for removal of subjects from the study:
Only dead or moribund fish will be removed from the test tanks.

5.7.2 Procedures for removal of subjects from the study:
Dip nets will be used to remove dead or moribund test fish from test tanks. Net(s) will be sanitized with disinfectant prior to and after each use.

5.7.3 Fate of removed study animals:
Dead and moribund fish removed from the study will be disposed of in an appropriate manner. Dead fish will be disposed of in an approved landfill or incinerated.

5.8 Concurrent/concomitant medications/therapies:
There will be no concurrent/concomitant medication/therapy administered during the course of the study.

5.9 General management practices:

5.9.1 Site visits:
Ideally, the Study Director, or his designee, will be at the study site at least 1 d prior to initiating the treatment phase of a study, and during the initial portion of the treatment period. The on-site Investigator (i.e., the facility manager or assistant manager, or qualified researcher) or their designee will be at the study site prior to and during the entire study. The Study Monitor, (a qualified Fish Health Biologist) will also conduct a site visit prior to initiating the study to perform a fish health evaluation on fish from the reference population. If the Study Monitor can not make a site visit, a "site-visit" will be made by phone, and fish samples from the reference population will be collected by the Study Director, Investigator or their designee, and sent to the Study Monitor for a fish health evaluation.

5.9.2 Data Collection:
The Study Director and on-site Investigator will assign study personnel to collect data. Study personnel responsible for collecting data or measuring

water quality parameters will be adequately trained by the Study Director or his designee.

5.9.3 Frequency of monitoring water chemistry parameters:
Table 5.9.3 lists frequency and location that water chemistry parameters that will be measured during the course of the study. Water temperature and dissolved oxygen will be measured at least once daily. If measured only once daily, measurements will be made in the morning. If measured twice daily, measurements will be made at the beginning and end of each workday. Water hardness, alkalinity, and pH will be measured at least once during the study.

5.9.3.1 Parameters to be measured prior to initiation of study:
Prior to initiation of treatment, the following will be measured and documented: (1) number of rearing units used in the study; (2) number of treated/untreated units; (3) mean fish weight; (4) mean fish length; (5) rearing unit configuration (i.e. - circular, rectangular); (6) rearing unit dimensions; (7) rearing unit size (ft^3); (8) number of fish/test unit; and (9) water flow (gallons/minute). Flow index and density index values will be calculated based on the above measured parameters.

5.9.3.2 What, when and where parameters will be measured during study:
Table 5.9.3 describes when and where treatment parameters will be measured during the course of the study. Twice daily indicates at the beginning and end of the work day; daily indicates at the beginning of the work day; once during study indicates at the beginning of the study. Water quality measurements will be recorded on data collection forms.

Table 5.9.3A. Parameters/schedule/location for taking water quality measurements.

Parameter	Schedule	Location
Dissolved Oxygen	Daily	All test tanks; mid-depth; tail-end
Water Temperature	Daily	All test tanks; mid-depth; tail-end
Water Flow	Set once, inspected visually daily	At test unit source
pH	Once during the study	One test tank; surface; any location

Alkalinity (as $CaCO_3$)	Once during the study	One test tank; surface; any location
Hardness (as $CaCO_3$)	Once during the study	One test tank; surface; any location

5.9.3.3 Procedures and equipment for assessing treatment parameters:
Tables 5.9.3.3 lists the equipment and BFTC standard operating procedures to be used to measure water quality parameters. If equipment other that those listed below are used, the alternative equipment will be comparable and capable of producing similar results following instructions in the owners manual. No specialized equipment is needed to measure water flow. Water flow will be measured by collecting and measuring water in a suitable container for a period of time to determine flow in gallons or liters per minute.

Table 5.9.3.3. Equipment and Reference Material for Measurement of Treatment Parameters.

Parameter	Equipment	Reference
Dissolved Oxygen	YSI DO and water temperature model 55 or 95	SOP No. INST 103 or 120
Water Temperature	YSI DO and water temperature model 55 or 95	SOP No. INST 103 or 120
Water Flow	Stop watch and container such as a bucket, beaker, or graduated cylinder	n/a
pH	YSI Model 60 pH Meter	SOP No. INST 125
Water Hardness	HACH Co. digital titrator, titrant, and reagents	SOP No. INST 105
Alkalinity	HACH Co. digital titrator, titrant, and reagents	SOP No. INST 104

5.9.3.4 Calculations for derived data:
Water hardness is the only water quality parameter measured in which calculations of the derived data are necessary to determine

final concentration (measured as mg/L as $CaCO_3$) . The calculations are described in Sop 105.

5.9.4 Frequency of feeding:
Test fish will be fed 2 - 4 times daily during the study. Fish will not be fed the day they are transferred from original rearing tanks to test tanks. Feed brand, size, amount, and feeding frequency will documented and described in the final study report.

5.9.5 Frequency of monitoring and adjusting water flow:
Water flow will be adjusted at the beginning of the study to pre-determined flows to achieve and maintain the designated flow index. Typically, source water head pressure at the study site will be sufficient so that there should be only minor changes in flow rates to test tanks. Depending on how water inflow is plumbed into test tanks, water flow rates will be visually inspected daily. Once water flows have been set, spigot handles should be marked so that if they are altered, they can be realigned visually. Water inflow measurements and adjustments will be made if visual inspections warrant this action.

5.10 Environmental conditions:
Field efficacy studies will be conducted under similar environmental conditions as those in which test fish were originally reared. Typically, a single water source will supply the entire hatchery (i.e., study site) with water. Consequently, the same source of water used to rear fish will be used for the study. Environmental conditions and physical test tank location will be documented and described in the final report.

5.11 Tank Cleaning:
Tanks will be cleaned at least once daily. When cleaned only once daily, tanks will be cleaned at the beginning of the day. If cleaned twice daily, tanks will be cleaned at the beginning and end of each day. Tank cleaning will done by using a brush to resuspend uneaten fish food and fecal material. Standpipes will be loosened to draw re-suspended material down the drain. Water should be drawn down to a level no further than that which would not unduly stress fish. Standpipes will then be refitted into test tanks, and water levels will return to full depth.

5.12 Provisions for necropsy and disposal of expired test subjects:
In most cases, necropsies will not be preformed. Cause of death will be presumed to have been caused by the pathogen identified during the pre-study fish health evaluation. If a fish necropsy is deemed necessary, due to lesions or uncharacteristic disease signs, a necropsy will be preformed by a trained fish health biologist in an appropriate area on site following guidelines outlined in references such as Procedures for the Detection

and Identification of Certain Fish Pathogens, 3rd edition (Amos 1995; see Appendix V). If a trained fish health biologist is not available to perform the necropsy, fish or fish tissue samples will be shipped overnight to trained fish health biologist (i.e., the Study Monitor), and a necropsy will be performed at their facility. Fish used in a necropsy will be disposed of in an approved landfill, or in a manner similar to that described in this protocol for disposal of dead test fish.

5.13 Fate of living test animals after study completion:

Test fish that had been treated with florfenicol medicated feed still living at the end of the study will not be slaughter, nor will harvest-able fish be stocked, until after the 21 d proposed investigational withdrawal period.

5.14 Test unit configuration:

Configuration of test tanks could be different at each study site. The Study Director or his designee, and the on-site Investigator will discuss potential test unit configuration design. A test unit configuration design will be used to minimize variability among test units. The test unit configuration, as well as test unit dimensions, standpipe height, water depth, and test unit rearing volume will be documented and described in the final study report.

5.15 Owner consent:

Not applicable.

6 SPECIFICATIONS OF VARIABLES:

6.1 Primary variable to be measured for evaluating labeled claim:

Mortality will be the primary response variable to be measured for evaluating the label claim. The proposed label claim will read, in part "...to control mortality in a variety of fish species caused by pathogens susceptible to florfenicol...."

6.1.1 When primary variables will be assessed:

Mortalities (dead or moribund fish) will collected, counted, and recorded at least once daily (i.e., at the beginning of the day). If mortality data are collected twice daily, then mortality data will be collected once in the morning and once in the afternoon.

6.1.2 Procedures for assessing primary variable:

No special procedures are necessary for assessing the primary variable. Dead fish will include moribund fish. Moribund fish will be defined as, in the opinion of study personnel, those approaching death. All study

personnel involved in collecting mortality data will have had extensive previous experience.

6.1.3 Equipment used for assessing primary variable:

No specialized equipment will be required to assess the primary variable.

6.1.4 Calculation of derived data:

Daily mortality during the treatment and post-treatment periods will be summed to determine total mortality. Mortality will typically be expressed as percent total mortality, which will be calculated by dividing total mortality by the number of fish per tank at the start of the study. Percent total mortality will be transformed using an arcsine transformation (Zar 1984) before data are analyzed using a statistical test such as a t-test or ANOVA. Number of fish at the start of the study will be determined using one of the following procedures:

6.1.4.1 Sample counts

A routine hatchery procedure called "sample counts" will be used to determine fish size. A subsample of fish from the reference population will be weighed, counted and an estimated individual fish weight will be calculated. Then, based on calculated individual fish weight, a pre-determined amount (i.e., weight) of fish will be collected, weighed, and transferred to test tanks to achieve target fish numbers.

6.1.4.2 Actual counts

Two procedures will be used to determine actual counts.

Actual counts during allocation of fish to tanks: In this procedure, fish will be counted from the reference or study population into a bucket partially filled with water, then the contents of the bucket will be transferred into a test tank. This is a convenient and accurate procedure to use when the number of test fish/tank does not exceed 300 fish/tank.

Actual counts at the end of the study: In this procedure, which is better suited when transferring large numbers of fish to test tanks, an estimated number of fish will be transferred into a tank using the sample count procedure described in Section 6.1.4.1. At the end of the study, fish remaining alive in each test tank will be hand-counted. Results from hand-counting fish from test tanks will be added to the total mortality in each of the test tanks,

resulting in an accurate number of fish/tank at the start of the study.

6.1.5 Forms for retention of source data:
Forms to be used for recording source data will provide space to record data for each test tank for each day of the study. There will be sufficient space on the form so that each entry may be printed clearly and legibly. Space will be provided for the initials of the individual recording data.

6.1.6 Name(s) and address(es) of outside labs used for analysis:
No outside labs will be used for analysis of the primary variable.

6.2 Other variables to be recorded during the study:
No other variables critical to addressing the study objective will be recorded. Other variables to be recorded during the study, such as water quality parameters, have been described elsewhere in the protocol (i.e., Section 5.9.3: Frequency of monitoring water chemistry parameters).

6.2.1 When other variables will be assessed:
n/a

6.2.2 Procedures for assessing other variables:
n/a

6.2.3 Equipment used to assess other variables:
n/a

6.2.4 Calculation of derived data:
n/a

6.3 Adverse reactions:
Adverse reactions by test fish to the florfenicol-medicated feed will be documented on the appropriate form or in a log book. The Study Director, Investigator, and Study Monitor should all be informed about the adverse reactions. This information will also be described in the final study report.

6.4 Study facilities:
The name and address of each study facility (i.e., the hatchery or research center where a clinical field efficacy study takes place) will be documented and described in the final study report. A number of locations, including federal, state, private, and tribal hatcheries, are potential study facilities. All study facilities will be suitable for conducting adequate and well-controlled pivotal field efficacy studies.

6.4.1 Containment equipment:

Test fish will be contained in a suitable fish tank or raceway. Fish tanks will be constructed of fiberglass, aluminum, or cement. Raceways will typically be constructed of cement. Type and/or size of containment equipment should not be of critical importance to the successful completion of a pivotal field efficacy study. The containment equipment (i.e., the test unit or test tank) used in each study will be documented and described in the final study report.

6.4.2 Lighting equipment:

No specialized lighting equipment will be used. Photoperiod will be similar among all test units at a particular facility, but may differ from facility to facility. When studies are conducted out-doors, no artificial light will be used. When studies are conducted in-doors, artificial lighting will supplement natural light.

6.4.3 Heating equipment:

No specialized heating equipment will be used.

6.4.4 Cooling equipment:

No specialized cooling equipment will be used.

6.4.5 Feeding equipment:

Typically, fish will be fed by hand. However, automatic feeders, such as Ziegler belt feeders, may be used. Feeding methods and equipment used will be described in the final study report.

6.4.6 Watering equipment:

Water inflow into test tanks will be gravity-fed or pumped. No other specialized watering equipment will be used.

6.4.7 Ventilation equipment:

No specialized ventilation equipment will be used:

6.4.8 Space allocation of test units:

Space allocation of test units will be sufficient so that study personnel can adequately feed fish and collect data. To ensure minimizing variability between test units, all test units will typically be positioned side-by-side in the same physical location.

6.4.9 Pasture allocation:

Not applicable

6.4.10 Facility diagram:
The layout of the complete study facility will not have an impact on study results. Consequently, a facility diagram for each study site will not be documented or included in the final study report. However, a diagram of the specific study layout will be included in the final study report.

6.5 Experimental diets:

All diets used in studies will be commercially available diets. Medicated feed will be top-coated at the BFTC in a 1.5 ft^3 capacity Marion Mixer model SPS 1224 (Marion Mixers, Inc., Marion, IA). Mixer operations will be done according to SOP No. INST 126. Unmedicated feed will be top-coating using the following procedure: (1) unmedicated feed will be poured in to the mixer; (2) the mixer will be turned on; (3) Aquaflor® will be added to the unmedicated feed and allowed to mix; (4) if a dry feed is used, approximately 0.5% (w/w) commercially available fish oil will be sprayed onto feed. Medicated feed may also be top-coated on feed or incorporated in feed by a licensed feed manufacturer. Unmedicated feed fed to control test fish during the treatment period will be the same type of feed fed to treated fish. During the post-treatment period, all fish will be fed unmedicated feed normally used at the facility where the study is being conducted.

6.5.1 Drug concentration of commercial diet:

6.5.1.1 Assayed drug concentrations:

Aquaflor® will be a 50% florfenicol premix. Feed will be mixed with different amounts of Aquaflor® to achieve the desired drug concentration. The drug concentration in the feed will be calculated and assayed analytically. Final drug concentration in the feed (measured in feed samples collected during the treatment period) will be documented and included in the final study report.

6.5.1.1.1 Drugs to be assayed in each treatment group:
Florfenicol

6.5.1.1.2 Anticipated analytical variation and assay limits:
Refer to the USGS, UMESC, LaCrosse, WI for anticipated analytical variation and assay limits.

6.5.1.1.3 Analytical method:

Refer to the USGS, UMESC, LaCrosse, WI for analytical methods.

6.5.1.1.4 Analytical Laboratory:
United States Geological Service
Upper Midwest Environmental Sciences Center

6.5.1.1.4.1 Address:
2630 Fanta Reed Road
LaCrosse, WI, 54603

6.5.1.1.4.2 Telephone Number:
608-781-6296 (Phone number of Chue Vue, Research Chemist, USGS, BRD, UMESC, LaCrosse WI)

6.5.1.1.5 Number of assay replicates:
Three samples will be collected from each lot of medicated feed used during the study. One sample will be collected at the beginning of the treatment period, one during the middle of the treatment period, and one at the end of the treatment period. One unmedicated feed sample will be collected at the beginning of the treatment period. Each sample will be assayed in triplicate. If a different unmedicated feed is used during the post-treatment period, one sample of second unmedicated feed used in the study will also be collected.

7 DATA ANALYSIS:

7.1 Define the experimental unit:
The experimental unit will be the test tank.

7.2 Define the number of replicates per treatment:
There will be a minimum of three replicates per treatment group.

7.3 Define statistical methodology:

7.3.1 Null hypothesis:
H_o : $u_1 = u_2$; Mean percent total mortality caused by pathogens susceptible to florfenicol is equal between groups of test fish treated with 10 mg florfenicol per kg fish body weight per day for 10 consecutive days, and groups of test fish that receive unmedicated feed.

7.3.2 Alternate (research) hypothesis:
H_a: $u_1 \leq u_2$; Mean percent total mortality caused by pathogens susceptible to florfenicol will be less in groups of test fish treated with 10 mg florfenicol per kg fish body weight per day for 10 consecutive days, than in groups of test fish that receive unmedicated feed.

7.3.3 Assumptions:

1) Two normally distributed populations.
2) Equality of variances is known.
3) Independent random samples of size n_1 and n_2.

7.3.4 Biostatistical procedures used:
If two treatment groups are used, an independent t-test will be used to detect differences in mean percent total mortality between treated and untreated groups of test fish. If more than two treatment groups are used, an analysis of variance will be used to detect difference in mean percent total mortality among the treatment groups. If differences are detected, a multiple comparison test, such as a Tukey multiple comparison test will be used to determine where differences exist. Where differences are stated to be significant, a level of $p \leq 0.05$ is implied. Mean percent total mortality will be transformed using an arcsine transformation prior to analyses.

7.3.5 Statistical data software to be used:
The statistical software package to be used will be SYSTAT (SPSS 1998), SigmaStat (SPSS 1997), or equivalent.

7.4 Define how the statistical results will be used to draw conclusions about the study's objective:

When there are two treatment groups in the study: If mean percent total mortality in the untreated groups is higher than mean total mortality in the treated groups, and the calculated *p* value is less than 0.05, then the conclusions drawn will state that the florfenicol medicated feed treatment regime used was effective in controlling mortality caused by pathogens susceptible to florfenicol in the test fish species used. If mean percent total mortality in untreated groups is higher than the mean total mortality in treated groups, but the calculated *p* value is greater or equal to 0.05, or if the mean percent total mortality in untreated groups is less than or equal to the mean total mortality in treated groups, then the conclusion drawn will state that the florfenicol medicated feed treatment regime used was not effective in controlling mortality caused by pathogens susceptible to florfenicol in the test fish.

When there are more than two treatment groups in the study: The same approach as described above will be used to draw conclusions about the study objective when comparing mean percent total mortality in two or more treated groups with mean percent total mortality in the untreated group. In addition, the same approach will be used to determine whether a difference in treatment efficacy exists between treatment groups.

8 ANALYTICAL METHODS:

8.1 Describe the analytical measurement to be made and the relevance to the protocol objective:

A high pressure liquid chromatography method will be used to confirm the concentration of florfenicol in medicated feed. This methodology has been developed or adapted for use by the staff at the UMESC. The sample preparation and analytical method are described in the UMESC SOP No. CAP 423.2 entitled "Determination of Florfenicol in fish Feed." Analytical verification of the concentration of florfenicol in the medicated feed is important to ensure that the actual treatment dosage administered to treated test fish is within acceptable assay limits of the target dosage established by FDA (Title 21 Code of Federal Regulations 558).

8.2 Specify the analytical plan to be used for the protocol measurements:

8.2.1 An abstract of the method:

This information is on file at the UMESC, La Crosse, Wisconsin, and is available upon request.

8.2.2 Description of procedures for sample selection, preparation, and storage:

Three feed samples will be collected and placed in properly labeled plastic zip-lock bags. One sample will be collected at the beginning of the of the treatment period, one sample will be collected during the middle of the treatment period, and one sample will be collected at the end of the treatment period. Samples will be stored in a freezer until shipped overnight in coolers containing freezer packs to the UMESC. Upon receipt of samples at the UMESC, samples will be visually inspected for damage, and placed in a freezer until analyzed.

8.2.3 Evidence of methods validation:

This information is on file at the UMESC, La Crosse, Wisconsin, and is available upon request.

8.2.4 Description of validation method plan when method is being developed for the study:

The analytical method used to verify florfenicol concentrations in feed was developed prior to the beginning of florfenicol-medicated feed field efficacy trials.

8.2.5 Quality control procedures for the method and criteria used to assess analytical results:

This information is on file at the UMESC, La Crosse, Wisconsin, and is available upon request.

8.3 Relevant scientific literature supporting the use of the analytical method for the intended measurements:

This information is on file at the UMESC, La Crosse, Wisconsin, and is available upon request.

8.4 Certification that all needed validations will be done before the initiation of the study:

This information is on file at the UMESC, La Crosse, Wisconsin, and is available upon request.

9 STUDY LOCATIONS:

A list of potential study sites is in the supplemental USFWS Compassionate Florfenicol INAD exemption protocol #10-697. If a study is to be conducted at a facility not listed in protocol #10-697, then the appropriate individuals at FDA and Schering-Plough Animal Health will be notified, and the study site information will be documented and described in the final study report.

10 PERSONNEL:

The Study Director or his designee has had past experience conducting pivotal field efficacy trials using medicated feed. The Study Monitor and Investigators will have had, in many cases, past experience conducting pivotal field efficacy trials. Other study personnel will typically be hatchery biologists or technicians fully capable of collecting and recording mortality data, measuring water temperature and DO concentration, and cleaning and feeding fish. It is not anticipated that study participants will be debarred from participating in a clinical field efficacy trial. However, if this is the case, information related to their debarment will be documented and described in the final study report. A copy of each study participants curriculum vitae will be included in the final study report.

10.1 Investigators, study monitor and fish health biologists involved in proposed field based clinical efficacy trials:

The names of the Investigators and Study Monitors (i.e., fish health biologists) will depend on the location of the study site. Potential study sites are in different regions of the USFWS, in different states, or are different private or tribal hatcheries or research facilities. Investigators and Study Monitors will be fully

capable of fulfilling their study responsibilities. The names of the Investigators and Study Monitors will be included in the final study report.

10.2 Other personnel involved in study:

Other personnel involved in studies will be documented and their name and study function will be included in the final study report.

11. COLLECTION AND RETENTION OF SOURCE DATA:

All source data, including those produced electronically, and a copy of all applicable reports will be retained at the Service's National INAD Office (NIO) in Bozeman, MT in a secure area which protects the source data and records from deterioration, destruction, tampering, and vandalism in accordance to Section D-4 paragraphs (a) and (b) of the Conduct of Clinical Investigations: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Studies (dated Oct. 1992 or later - see Attachment II).

12 ADDENDUM/DEVIATIONS TO THE PROTOCOL:

12.1 Protocol addendums:

Protocol addendums will attached to the original version of study protocol FLOR-01-EFF, Efficacy of florfenicol medicated feed for control of mortality associated with pathogens susceptible to florfenicol a variety of fish species. If a protocol addendum is sent to FDA Center for Veterinary Medicine, a cover letter will accompany the addendum, referencing the submittal date of the above protocol, describing the purpose of the addendums. Addendums and copies of the cover letter sent to FDA will be kept with the original study protocol in Appendix V.

12.2 Protocol amendments:

A signed copy of the Study Protocol will be retained by the on-site Investigator during the pre-treatment, treatment, and post-treatment periods. At any time before a study begins, desired changes to the Study Protocol should be brought to the attention of the Study Director, Investigator, and Study Monitor. The desired changes will be documented. Documentation will include date of amendment, reason for change, and the amended text, and kept with the original study protocol in Appendix V. The amendment will be signed by the Study Director. Copies of the signed amendment will be attached to the original Study Protocol. All study personnel will be made aware of protocol amendments prior to starting a study.

12.3 Protocol deviations:

Deviations from the established Study Protocol, that could potentially negatively impact the outcome of the study, will be documented in the final study report. If deviations occur, the Study Director, Investigator, and Study Monitor should be contacted immediately for advice. If possible, protocol deviations will be

documented fully when they occur, accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation. If deviation statements are entered in log books as raw data, they will be signed and dated.

13 DRUG DISPOSITION/ANIMAL ACCOUNTABILITY/FEED DISPOSITION/FEED ACCOUNTABILITY:

Unused drug remaining at the end of a study must be disposed of in an approved landfill or by incineration, unless there is an immediate need for unused florfenicol-medicated feed for use in another pivotal field efficacy trial. If this is the situation, then it is possible that the feed may be used int the other pivotal field efficacy trial rather than disposed.

14. REFERENCES

Alderman, D.J. 198. Fisheries chemotherapy: a review. In: Recent Advances in Aquaculture, Vol. 3 (ed by J.F. Muir and R.J. Roberts) pp. 1-61. Croom Helm, London

Amos, Kevin H. , editor. 1985. Procedures for the Detection and Identification of Certain Fish Pathogens. Third Edition. Fish Health Section, American Fisheries Society. Corvallis, Oregon.

Bjorndal, T. 1990. The economics of salmon aquaculture. Blackwell Scientific Publications, Oxford.

Clark, R., and D. Scott. 1989. An overview of world salmon production and recent technology developments. Bulletin of the Aquaculture Association of Canada. 4:31:48

Ellis, A.E. 1989. Use of vaccines in controlling fish diseases. Developmental and Comparative Immunology. 13:399

Frerichs, G.N., and R.J. Roberts. 1989. The bacteriology of teleosts in: Fish Pathology, 2nd edition (ed. By R.J. Roberts), pp. 239-319. Balliere Tindall, London

Fukui, H., Y. Fujihara, and T. Kano. 1987. In vitro and in vivo antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against fish pathogens. Fish Pathology. 22:201-207

Hastein, T. 1988. Disease control through management practices. In: Proceedings of the Aquaculture International Congress, P. 39. Aquaculture International Congress, Vancouver, B.C.

Horsberg, T.E., K.A. Hoff and R. Nordmo. 1996. Pharmacokinetics of florfenicol and its metabolite florfenicol amine in Atlantic salmon. Journal of Aquatic Animal Health 8:292-301

Klontz, G.W. 1987. Control of systemic bacterial diseases in salmonids. Salmonid. 11:5-13

McCarthy, D.H. and R.J. Roberts. 1980. Furunculosis of fish - the present state of our knowledge. In: Advances in Aquatic Microbiology (ed. by M.A. Droop and H.W. Jannasch, pp.293-341. Academic Press, London

Munro, A.L.S. and R.J. Roberts. 1989. The aquatic environment. In: Fish Pathology, 2nd edition (ed. by R.J. Roberts), pp.1-11. Balliere Tindall, London

Nagata, T. and H. Oka. 1996. Detection of residual chloramphenicol, florfenicol, and thiamphenicol in yellowtail fish muscles by capillary gas chromatography-mass spectrometry. Journal of Agriculture Food Chemistry. 44:1280-1284

Nordmo, R., K.J. Varma, I.H. Sutherland, and E.S. Brokken. 1994. Florfenicol in Atlantic salmon, *Salmo salar* L.; field evaluation of efficacy against furunculosis in Norway. Journal of Fish Diseases. 17:239-244

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler and J.R. Leonard. 1982. Fish Hatchery Management. United States Department of the Interior, U.S. Fish and Wildlife Service, Washington, DC., 517 pp.

Powers, T.E., K.J. Varma and J.D. Powers. 1990. In F.Simon (editor). Abstracts of the 4th Congress of European Associations of Veterinary Pharmacology and Toxicology, Budapest, Aug 28-Sep 2, 1988. Vol. 1, University of Veterinary Science, Budapest, 1990, pp. 3-6

Samuelsen, O.B., B Hjentnes and J. Glette. 1998. Efficacy of orally adminstered florfenicol in the treatment of furunculosis in Atlantic salmon. Journal of Aquatic Animal Health 10:56-61

SPSS. 1997. SigmaStat 2.03 statistical software. SPSS, Inc., Chicago, Illinois.

SPSS. 1998. SYSTAT 8.0 statistical software. SPSS, Inc., Chicago, Illinois.

Yasunaga, N., and S. Yasumoto. 1988. Therapeutic effect of florfenicol on experimentally induced pseudotuberculosis in yellowtail. Fish Pathology 23:1-5

Zar, J. H. 1984. Biostatistical analysis, 2nd Edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

FORMS

1. Environmental and culture conditions prior to treatment initiation
2 Daily mortality
3. Daily dissolved oxygen
4. Daily water temperature
5. pH and hardness

APPENDICES:

I. Florfenicol MSDS

II. Standard fish health diagnostic scheme to confirm fish pathogens susceptible to florfenicol.

III. Investigational Withdrawal Period

IV. Procedures for necropsy.

V. Amendments and Addendums to the study protocol.

Form 1. **Test Site, Species Tested, Environmental and Culture Conditions, and Water Quality Parameters**

Test Site: _____

_____

_____

phone ( )_____fax ( )_____

Study Monitor _____ Study Investigator _____

Other Study Personnel _____

Florfenicol Lot Number _____ Test Site Elevation: _____

Fish Species: _____ Water Source: _____

% Body Weight fish to be fed at: _____

Feed Brand, Type, and Size: _____

Fish Source (originating facility/body of water): _____

Fish Lot Number: _____ Fish Length (in): _____ Fish Weight (g): _____

Test Unit Type (e.g. fiberglass/circular, aluminum/trough, concrete/raceway): _____

Test Unit Dimensions (ft) : _____ Standpipe Height (in): _____

Test Unit Volume (cu. ft): _____ Test Unit Volume (gal): _____

Number of Control Test Units: _____ Number of Treated Test Units: _____

Number of Fish/Test Unit: _____ Water Flow per Test Unit (gpm): _____

Flow Index: _____ Density Index: _____

NOTE:

It is assumed that test conditions are identical for each test unit in the study. If not, please specify any irregularities on a separate sheet and attach to this form.

Study Director: _____

Signature and Date

Investigator: _____ Study Monitor: _____

Signature and Date Signature and Date

Form 2. Daily Mortality Record for Pivotal Studies Evaluating the Efficacy of Florfenicol Medicated Feed

Daily Mortality

	Study day	Date	Rearing Unit #	Rearing Unit #	Rearing Unit #	Rearing Unit #	Rearing Unit #	Rearing Unit #	Initials
Treatment Period	1								
	2 trt 1								
	3 trt 2								
	4 trt 3								
	5 trt 4								
	6 trt 5								
	7 trt 6								
	8 trt 7								
	9 trt 8								
	10 trt 9								
	11 trt 10								
Post - Treatment Period	12								
	13								
	14								
	15								
	16								
	17								
	18								
	19								
	20								
	21								
	22								
	23								
	24								
	25								

NOTE: Mortality should be collected once daily.

Study Director _____ Date _____

Study Monitor _____ Date _____

Investigator _____ Date _____

Form 3. Daily Record for Dissolved Oxygen Levels in Pivotal Studies Evaluating the Efficacy of Florfenicol Medicated Feed

Daily Dissolved Oxygen (mg/L)

Study Day	Date	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Initials
1		/	/	/	/	/	/	/
2		/	/	/	/	/	/	/
3		/	/	/	/	/	/	/
4		/	/	/	/	/	/	/
5		/	/	/	/	/	/	/
6		/	/	/	/	/	/	/
7		/	/	/	/	/	/	/
8		/	/	/	/	/	/	/
9		/	/	/	/	/	/	/
10		/	/	/	/	/	/	/
11		/	/	/	/	/	/	/
12		/	/	/	/	/	/	/
13		/	/	/	/	/	/	/
14		/	/	/	/	/	/	/
15		/	/	/	/	/	/	/
16		/	/	/	/	/	/	/
17		/	/	/	/	/	/	/
18		/	/	/	/	/	/	/
19		/	/	/	/	/	/	/
20		/	/	/	/	/	/	/
21		/	/	/	/	/	/	/
22		/	/	/	/	/	/	/
23		/	/	/	/	/	/	/
24		/	/	/	/	/	/	/
25		/	/	/	/	/	/	/

NOTE: Dissolved Oxygen should be collected twice daily, once in the morning and again in the afternoon.

Study Director_____ Date _____ Study Monitor _____ Date_____

Investigator _____ Date _____

Form 4. Daily Record for Water Temperature in Pivotal Studies Evaluating the Efficacy of Florfenicol Medicated Feed

Daily Water Temperature (°C)

Study Day	Date	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Rearing Unit # am / pm	Initials
1		/	/	/	/	/	/	/
2		/	/	/	/	/	/	/
3		/	/	/	/	/	/	/
4		/	/	/	/	/	/	/
5		/	/	/	/	/	/	/
6		/	/	/	/	/	/	/
7		/	/	/	/	/	/	/
8		/	/	/	/	/	/	/
9		/	/	/	/	/	/	/
10		/	/	/	/	/	/	/
11		/	/	/	/	/	/	/
12		/	/	/	/	/	/	/
13		/	/	/	/	/	/	/
14		/	/	/	/	/	/	/
15		/	/	/	/	/	/	/
16		/	/	/	/	/	/	/
17		/	/	/	/	/	/	/
18		/	/	/	/	/	/	/
19		/	/	/	/	/	/	/
20		/	/	/	/	/	/	/
21		/	/	/	/	/	/	/
22		/	/	/	/	/	/	/
23		/	/	/	/	/	/	/
24		/	/	/	/	/	/	/
25		/	/	/	/	/	/	/

NOTE: Water temperature should be collected twice daily, once in the morning and again in the afternoon.

Study Director_____ Date _____ Study Monitor _____ Date_____

Investigator _____ Date _____

Form 5. Record of Water Hardness, Alkalinity, and pH in Pivotal Studies Evaluating the Efficacy of Florfenicol to Control Mortality.

Date	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	pH	Initials

Study Director _____ Date _____

Study Monitor _____ Date _____

Investigator _____ Date _____

Raw Data: Water hardness and alkalinity - record the number digits on the digital titrator that were required to get the correct color change in the water sample, and show calculations used to derive concentration (mg/L $CaCO_3$).

Hardness #1

Alkalinity #1

Hardness #2

Alkalinity #2