COLLECTION OF PIVOTAL FIELD EFFICACY DATA AND TISSUE RESIDUE DEPLETION DATA IN SUPPORT OF A NEW ANIMAL DRUG APPROVAL FOR FLORFENICOL, OXYTETRACYCLINE, AND CHLORAMINE-T

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FUNDING REQUEST: $16,615

OBJECTIVES:

1. To evaluate the effectiveness of florfenicol and oxytetracycline to control mortalities caused by *Flavobacter columnare* in selected cultured fish and to evaluate the effectiveness of chloramine-T to control mortalities caused by bacterial gill disease in selected cultured fish.

2. To collect tissue residue depletion data for florfenicol, oxytetracycline, and chloramine-T in selected cultured fish.

JUSTIFICATION

Maintenance and/or propagation of fishes, both public and private, is dependent upon the use of drugs to maintain fish health and product quality. Currently, only four therapeutants and a single anesthetic are approved for aquaculture use in the United States. This situation is complicated by the fact that use of these compounds is severely restricted by species, life stage, and disease organism. Fish propagation and maintenance in captivity desperately needs approved drugs to sustain production for sport fisheries, restoration/recovery programs, and food production. The U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine has recognized that provisions need to be developed to allow the use of certain drugs in aquaculture programs until full drug approvals, New Animal Drug Applications (NADAs), can be obtained. The primary provision for this purpose has been the granting of compassionate Investigational New Animal Drug (INAD) exemptions. Compassionate INADs are granted for purposes related to the health and well being of animals, and allow fishery managers/producers to treat large numbers of animals with unapproved drugs during the period of time that dose-response data are still being collected. This is in marked contrast to "standard" INADs that allow drug/pharmaceutical companies to conduct clinical laboratory investigations on a relatively small number of animals for the sole purpose of obtaining data that can be used to obtain an approved drug label. In the case of standard INADs, *all data* must be obtained prior to drug use in the field. Compassionate INADs were actually developed for, and by, the aquaculture industry.

Numerous requirements must be met for the establishment and maintenance of aquaculture INADs. FDA reviews study protocols, authorize specific conditions of use, and closely monitor all drug use under INAD exemptions. Data recording, analysis, and reporting are required on a regular basis. **INAD exemptions are granted by FDA with the expectation that meaningful data will be generated from INAD studies, and that such data will be used to support an NADA.**

In response to this national resource issue, the U.S. Fish and Wildlife Service (USFWS) has assumed a leadership role by developing a program to insure compliance with all regulatory issues related to drug testing for aquatic species. In 1994 the USFWS established a National INAD Office (NIO) in Bozeman, Montana. The NIO was created to oversee the USFWS's INAD program and to coordinate all USFWS INAD activities. It was also established to assure that progress was made toward both maintenance of INADs and approved drug labels. During the last several years, the NIO has had the opportunity to review/analyze all of the data generated by USFWS facilities under INAD exemptions. In virtually all cases, data collected from field stations has not been pivotal data, but rather what is termed "ancillary or supporting data." While such information is in fact useful to FDA (for the purpose of writing drug labels), it will not support full drug approval. For example, none of the 187 trials on chloramine-T to treat bacterial gill disease in various cultured fish species analyzed by the NIO during a 12 month period contained pivotal data. A NADA package must contain at least a certain minimum amount of pivotal data.

Therefore, it is imperative that studies be performed using selected coolwater and warmwater species in order to add these fish groups to the label under the crop grouping concept. Without the collection of pivotal data, INADs become little more than "use permits" that will not be renewed indefinitely.

Based on these findings, it has become obvious to both the NIO and the FDA that the only path by which pivotal data will be generated is if someone puts forth the effort to design and initiate studies to provide exactly such information. Treating fish as the need happens to arise will simply never provide the pivotal data necessary to support a NADA. It is imperative that some agency or organization take additional action to insure that pivotal field efficacy data are collected. The NIO is a logical choice to conduct/coordinate such an effort, as it has both the expertise and facility contacts required. The NIO currently oversees all INAD-approved drug use by all USFWS facilities, as well as all INAD-approved drug use at approximately 150 state, tribal, and private aquaculture facilities (as a result of the USFWS's National INAD Program). FDA currently requires that dose titration studies be followed up by clinical field trials from at least three different geographical locations.

The Iowa Department of Natural Resources (IDNR) has been asked to cooperate with the NIO and the Upper Midwest Environmental Sciences Center (UMESC) at La Crosse, Wisconsin to help generate the necessary efficacy and tissue residue depletion data. In order to accomplish this task, the IDNR will make available the necessary facilities, fish, and personnel. The primary testing location will be the Rathbun Fish Culture Research Facility; however, other IDNR sites may participate if required. Three drugs, florfenicol, chloramine T, and oxytetracycline, and two fish species, walleye and channel catfish, were selected for this study.

1. Florfenicol is a potent, broad spectrum antibacterial agent with bacteriostatic properties. 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. Florfenicol (Aquaflor®) is currently approved in Canada for the control of furunculosis in Atlantic salmon.

Bacterial diseases remain a major problem in aquaculture and account for significant losses of fish. Florfenicol has great potential for treatment of infectious fish diseases. Thus, florfenicol 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.

2. Oxytertacycline (trade name Terramycin) is a quaternary salt with broad spectrum antibacterial properties. The drug is commonly used in medicated feed for treating flavobacteriosis in fish.

3. Chloramine-T is a white crystalline powder with the chemical name sodium p-toluenesulphonchloramide. The drug is commonly used as a bath treatment for external *Flavobacter* infections such as bacterial gill disease.

The purpose of these field based clinical efficacy trials is to evaluate the efficacy of florfenicol medicated feed treatment at a dosage of 10 mg of active drug per kg of fish per day for 10 days and oxytetracycline medicated feed treatment at a dosage of 2.5-10.0 g of active drug per 100 lb (454 kg) of fish per day for 10 days to control mortality in selected fish species caused by *Flavobacter columnare;* and to evaluate the efficacy of chloramine-T at up to 20.0 mg/L in a water bath to control mortalities in selected fish caused by bacterial gill disease. In addition, the time necessary for these drugs to deplete from fish tissue to an accepted safe level will be evaluated.

RELATED CURRENT AND PREVIOUS WORK

Much data are required by FDA for a drug to achieve NADA approval and the work proposed here is only a portion of the necessary data. Data have been and is currently being generated as part of the total drug package that will be submitted; however, to date, no field efficacy and tissue residue data have been generated for the selected disease and species proposed in this study.

Literally millions of dollars are being spent to administer INAD programs collecting ancillary efficacy data and to generate toxicity, residue, human food safety, and environmental safety data required for a NADA.

However, none of these monies are being directed towards generating pivotal field efficacy data, which is also a critical requirement for a NADA. At this point, the success of the entire INAD/NADA effort hinges on collection of pivotal field efficacy data. A relatively small amount of money, directed specifically for the purpose of obtaining such efficacy data, will insure the ultimate success of the INAD/NADA process.

ANTICIPATED BENEFITS

Data from the studies will help complete the necessary data packages required by FDA for approval and labeling of these drugs. Once a NADA is secured, these drugs will be legal to use in aquaculture in the United States. The cost benefits of this study proposal are tremendous if one takes into account the funding level already committed to the INAD/NADA process through the cooperative federal/state aquaculture drug approval partnership project, the USFWS's own INAD program, and other state and private INAD programs.

This study has national significance in that an approved drug label for the use of these drugs in fish will benefit the entire aquaculture community including federal, state, and private aquaculture interests located throughout the United States. This study will provide direct benefits to the 37 states involved in the federal/state aquaculture drug approval partnership project to obtain approval for drugs for public fish production that is based upon a Memorandum of Understanding between the International Association of Fish and Wildlife Agencies (IAFWA), the U.S. Geological Service's Biological Resource Division, and the USFWS. In the long-run, it will also directly benefit the remaining 13 states in the US. Virtually all recreational fish species that have a culture/propagation component to their management program will benefit.

All recreational fisheries opportunities that are in part based on culture/propagation for stock enhancement will benefit from this study. While in certain parts of the country there are indeed naturally reproducing, wild (not necessarily native) fish populations that support recreational opportunities without stock enhancement, this situation is certainly the "exception rather than the rule." From Pacific salmon on the west coast, to trout, walleye, and muskellunge in the midwest, to catfish and striped bass in the southeast, to Atlantic salmon on the east coast, many recreational fishing opportunities are dependent upon culture/propagation programs. As the popularity of fishing and associated recreation continues to rise in this country, the pressure on existing fish populations will also continue to increase.

PROCEDURES

The following study procedures are a condensation of the more detailed protocols that will be used to guide the studies. A copy of these detailed protocols is available upon request.

Effectiveness of florfenicol, oxytetracycline, and chloramine-T (Objective 1)

Florfenicol

Walleye *(Stizostedion vitreum)* and channel catfish *(Ictalurus punctatus)* will be tested at the Rathbun Fish Culture Research Facility (RFCRF) and/or another IDNR facility if necessary. A listing of identified potential study sites is presented in the facilities section. The pathogen to be tested will be *Flavobacter columnare.*

Studies will typically consist of replicated treated groups and replicated non-treated groups. All fish for a particular study will be from the same lot. Study fish will either be from a single rearing unit, or from multiple rearing units managed under the same environmental and culture conditions. Treated groups will receive florfenicol-treated feed at a dosage of 10 mg active drug per kg fish per day for 10 days. Control fish will receive non-medicated feed at the same feed rate as treated fish. All treatments will be conducted in at least triplicate.

Fish used in studies will typically be characterized as "early life stages," less than one year old and between 2.5-20.3 cm mean length. Test fish will be reared under standard hatchery conditions as described by Piper et al., 1981. A description of pre-study rearing parameters will be included in the final report detailing egg source, management practices, environmental conditions, and water quality

parameters. The number of test fish used in each study will be predicated on achieving a flow index and density index that approximates the normal production conditions under which the disease condition initially occurred. Entrance criteria for inclusion of specific fish lots in these studies will include: (1) study fish are diagnosed with *F. columnare* pathogens; (2) fish are known to be free of secondary disease pathogens that might mitigate drug eficacy; and (3) sufficient test units and fish are available to conduct studies in triplicate under production-like conditions. Diagnosis of the disease will be confirmed by a fish health specialist using accepted diagnosis techniques.

The experimental design used will be completely randomized. Test fish will be randomly placed in test units in such a manner as to minimize bias. It is anticipated that no blocking factor(s) will be used as all fish will be held under identical rearing/environmental conditions. All studies will be blinded so that only the principal investigator (or a single appointee of the principal investigator) has prior knowledge regarding experimental unit treatment condition. All other study participants will remain blinded to preclude potential bias in data collection. Test conditions will be nearly identical to conditions under which the fish became diseased. A 24-hour acclimation period before treatment may be used if fish are transferred from rearing units to test units.

The primary response variable in these studies will be mortality (e.g., dead fish). Mortalities will be removed, counted, and recorded daily, beginning the first day of the treatment period and ending 14-30 days post-treatment. Pre-study mortality will also be recorded for a period of 10 days prior to the initiation of the study. Secondary response variables will include observations of general fish behavior/condition, as well as complete pre- and post-study fish health evaluations to determine effects of florfenicol treatment.

The only analytical measurement required in these studies will be dose verification of florfenicol medicated feed to confirm target dosages. This work will be contracted to a certified analytical laboratory. Prior to initiation of treatment, the following will be measured: mean fish weight, mean fish length, rearing unit design, number of fish per test unit, and water flow. Water quality parameters to be measured will be: dissolved oxygen, water temperature, water flow, and pH.

The experimental design will test the hypothesis H_0: $u_1 = u_2$. Mortality caused by *F. columnare* is equal between fish treated with 10 mg florfenicol per kg fish per day for 10 days, and fish that receive unmedicated feed (H_0: u (mortality in treated fish) = u (mortality in non-treated control fish). The alternate hypothesis tested will be H_A: $u_1 \leq u_2$. Mortality caused by *F. columnare* will be lower among fish treated with 10 mg florfenicol per kg fish per day for 10 days, than fish that receive unmedicated feed (H_A: u (mortality in treated fish) $\leq$ u (mortality in non-treated control fish).

An independent t-test will be used to detect differences between treated and untreated fish with regard to total fish mortality per test unit. Where differences are stated to be significant, a level of $P \leq 0.05$ will be implied. Where variances are equal, results from the pooled variance t-test will be used. Where variances are unequal, results from the separate variances t-tests will be used. The separate variances t-test adjusts the degrees of freedom to account for unequal variances.

Oxytetracycline

Walleye *(Stizostedion vitreum)* will be tested at the RFCRF and/or another IDNR facility if necessary. A listing of identified potential study sites is presented in the facilities section. The pathogen to be tested will be *Flavobacter columnare.*

Studies will typically consist of replicated treated groups and replicated non-treated groups. All fish for a particular study will be from the same lot. Study fish will either be from a single rearing unit, or from multiple rearing units managed under the same environmental and culture conditions. Treated groups will receive oxytetracycline-treated feed at a dosage of 2.5-10.0 g active drug per 100 lb of fish per day for 10 days. Control fish will receive nonmedicated feed at the same feed rate as treated fish. All treatments will be conducted in at least triplicate.

Fish used in studies will typically be characterized as "early life stages;" less than one year old and between 2.5-20.3 cm mean length. Test fish will be reared under standard hatchery conditions as described by Piper et al., 1981. A description of pre-study rearing parameters will be included in the final

report detailing egg source, management practices, environmental conditions, and water quality parameters. The number of test fish used in each study will be predicated on achieving a flow index and density index that approximates the normal production conditions under which the disease condition initially occurred. Entrance criteria for inclusion of specific fish lots in these studies will include: 1) study fish are diagnosed with *F. columnare* pathogens; 2) fish are known to be free of secondary disease pathogens that might mitigate drug efficacy; and 3) sufficient test units and fish are available to conduct studies in triplicate under production-like conditions. Diagnosis of the disease will be confirmed by a fish health specialist using accepted diagnosis techniques.

The experimental design used will be completely randomized. Test fish will be randomly placed in test units in such a manner as to minimize bias. It is anticipated that no blocking factor(s) will be used as all fish will be held under identical rearing/environmental conditions. All studies will be blinded so that only the principal investigator (or a single appointee of the principal investigator) has prior knowledge regarding experimental unit treatment condition. All other study participants will remain blinded to preclude potential bias in data collection. Test conditions will be nearly identical to conditions under which the fish became diseased. A 24-hour acclimation period before treatment may be used if fish are transferred from rearing units to test units.

The primary response variable in these studies will be mortality (e.g., dead fish). Mortalities will be removed, counted, and recorded daily, beginning the first day of the treatment period and ending 14-30 days post-treatment. Pre-study mortality will also be recorded for a period of 10 days prior to the initiation of the study. Secondary response variables will include observations of general fish behavior/condition, as well as complete pre- and post-study fish health evaluations to determine effects of oxytetracycline treatment.

The only analytical measurement required in these studies will be dose verification of oxytetracycline medicated feed to confirm target dosages. This work will be done using an HPLC method developed by the staff at the UMESC. Prior to initiation of treatment, the following will be measured: mean fish weight, mean fish length, rearing unit design, number of fish per test unit, and water flow. Water quality parameters to be measured will be: dissolved oxygen, water temperature, water flow, and pH.

The experimental design will test the hypothesis H_0: $u_1 = u_2$. Mortality caused by *F. columnare* is equal between fish groups treated with oxytetracycline medicated feed at a rate of 2.5-10.0 g of active drug per 100 lb (454 kg) of fish per day for up to 10 days, and fish groups that receive unmedicated feed (H_0: u (mortality in treated fish) = u (mortality in non-treated control fish). The alternate hypothesis tested will be H_A: $u_1 \leq u_2$. Mortality caused by *F. columnare will* be lower among fish groups treated with oxytetracycline medicated feed fed at a rate of 2.5-10.0 g of active drug per 100 lb of fish for up to 10 days than among fish that receive unmedicated feed (H_A: u (mortality in treated fish) $\leq$ u (mortality in non-treated control fish).

An independent t-test will be used to detect differences between treated and untreated fish with regard to total fish mortality per test unit. Where differences are stated to be significant, a level of $P \leq 0.05$ will be implied. Where variances are equal, results from the pooled variance t-test will be used. Where variances are unequal, results from the separate variances t-tests will be used. The separate variances t-test adjusts the degrees of freedom to account for unequal variances.

Chloramine-T

Walleye (*Stizostedion vitreum*) will be tested at the RFCRF or another designated facility if necessary. Bacterial gill disease (BGD) will be the disease to be tested.

Fish will be held in a culture rearing unit until an epizootic of BGD occurs. Fish in the culture unit experiencing the epizootic will be gently herded from the effluent end to the head end of the culture unit using a seine. Fish will be subsampled by passing a dip net from the bottom of the culture unit to the surface. Three samples of four fish each, for a total of 12 fish, will be collected for disease diagnosis. A second group of fish will be subsampled and randomly assigned to test tanks where the chemical treatments will be administered. Disease diagnosis will be performed prior to initiation of treatments to confirm the presence of disease in the population. Fish placed in the treatment tanks will receive their first

treatment within 24 hr after diagnosis of the disease. No attempt will be made to acclimate the fish to test conditions, because they will be tested under physical conditions similar to those found in the culture unit from which they were removed.

Fish with BGD exhibit lethargic behavior, reject food, "ride high" in the water column, and orient toward the current (Lasee 1995). Fish exhibiting these behaviors will be diagnosed for the disease by microscopic observation of a gill arch (Lasee 1995). Prior to dissection, fish will be euthanized. The presence of filamentous bacteria will be a presumptive confirmation of BGD infection.

The exposure system will consist of 12 stainless steel or fiberglass tanks (provided by UMESC) covered by lids. The tanks used for treatments will vary in size from 15 to 100 L depending on the size of the fish (test volumes will be recorded on the appropriate data sheet). Each test tank will be supplied with a continuous flow of hatchery water at a rate (L/min) that provides approximately the same number of volume exchanges per day as was supplied to the culture unit from which the fish were removed (e.g. five tank exchanges/ day for each). Temperatures will be maintained within ±2°C of the culture unit water temperature.

Four groups of fish of equal numbers will be tested; three treatment groups (0, 10, and 20 mg/L) and an untreated control group. Each group will be tested in triplicate (three tanks). All test fish will come from the same culture unit experiencing the disease outbreak. The number of fish in each tank will be selected so that the density (number fish/volume or weight/volume) will be 20 ± 5% higher than the fish density in the culture unit where they were cultured prior to the disease outbreak (example: culture tank 1,000 g of fish/1000 L, test tank 120 g fish/100 L). The higher stocking density should reduce the chance of spontaneous remission (fish recovery from the disease usually due to improved water quality) of the disease in the test fish. Each test tank will have a minimum of five fish to ten fish depending on fish size. Higher densities will provide a worst case scenario and provide an extreme situation for the drug to control mortalities from flavobacteria infections.

Fish will be randomly assigned to each replicate (tank) of a group. The arrangement (order) of each treatment group in a test series will also be randomly determined. The chloramine-T treatments will be administered as a static bath.

The chloramine-T will be administered to the treatment tanks once every other day for a total of three treatments. The identity of treatment groups and exposure water samples will be blinded. During the static treatments, water flow to each tank will be shut off for the entire 60-minute exposure period. Aeration will be supplied to all tanks during the exposure period. Treatment solutions (0, 10, 20, and 30 mg/L) will be prepared by adding a calculated amount of chloramine-T dissolved in a known volume of water. The treatment solutions will then be added directly to each exposure tank and the exposure water will be mixed by gentle stirring. After the 60 minute exposure, the water flow to each tank will be restored at an increased rate (minimum of three volume replacements/five minutes) designed to rapidly flush the treatment solution from each tank over a 5-10 minute period. After the tank is flushed, the flow will be reset to the original pretreatment flow rate.

The chloramine-T exposure water will be sampled from each tank (one treatment tank will be sampled in triplicate) 10-20 minutes before termination of a treatment. Water samples (1-10 mLs) will be taken by pipette from the middle of the treatment unit at mid-depth. Chloramine-T concentrations will be verified in each treatment tank using a Hach Pocket Colorimeter® DPD test (CAP 415). The DPD test procedures outlined in CAP 415 will be followed for all chloramine-T analyses. Chloramine-T concentrations of ±10% of the expected treatment concentration will be considered acceptable.

All chemical treatments will be blinded. Test tanks will receive an identification number from 1-12 (representing controls and three treatment concentrations). Only the study director or his designee will know which treatment is associated with a specific treatment tank number. Treatment volumes of chloramine-T stock solution will be measured separately for each test tank and placed in a beaker with an identification number from 1-12 corresponding to the tank number designation. The prepared stock solution volumes will be diluted to a 500 mL total volume using hatchery water. Controls will receive 500 mL of hatchery water. An assistant will apply the treatments with no knowledge of the identity of the treatments because all treatment additions will be of the same volume and appearance.

Only numbers (1-12) will be used to identify vessels on the data sheets (treatment groups will be unknown). The only exception to this will be the recording of chloramine-T treatment concentrations (alphabetical letters will be used) on the chloramine-T analysis data sheets. Water samples for chloramine-T analysis will be placed in beakers by the study director or his designee (only personnel who will know the identity of the samples). The beakers will be labeled in a manner such that the person conducting the analysis will not be able to determine the origin of the samples (i.e. beakers labeled with a letter instead of a number). During the post-treatment phase of the study, fish will remain in the same exposure tanks and the same blinding procedures will be maintained.

Mortality is considered a dependent variable. Mortality will be defined as the cessation of opercular movement or the lack of response to gentle prodding. Cumulative mortality (summation of all mortalities up to the day of recording) will be recorded for each treatment replicate daily. The initial reading will be made approximately 24 hr after the first treatment. Mortality readings on treatment days will be taken prior to addition of the chemical. Dead fish will be removed from the tanks daily. Post treatment mortality data will be collected daily for up to 14 days following the final treatment (third treatment). Fish will be held in the treatment tanks during the post-treatment period.

The comparison of the percent moralities in the controls to the treatments will provide a measurement of the effectiveness (efficacy) of chloramine-T to control the disease. Water quality parameters (pH, temperature, or dissolved oxygen) that vary by more than 10% between the test tanks and the raceway during any treatment will be reported in the lab book.

The experimental design will test the hypothesis H_0: $u_1 = u_2$. Mortality caused by BGD is equal between fish groups treated with chloramine-T for three treatments, and non-treated fish groups (H_O: u (mortality in treated fish) = u (mortality in non-treated control fish). The alternate hypothesis tested will be H_A: $u_1 \leq u_2$. Mortality caused by BGD will be lower among fish groups treated with chloramine-T for three treatments than among non-treated fish (H_A: u (mortality in treated fish) $\leq$ u (mortality in non-treated control fish).

Mortality data will be analyzed by the study director or his designee. The study director or his designee will summarize the data (cumulative mortality) from each treatment through tabulation and descriptive statistics. Differences in mortality between test groups will be tested. A significance level of $P \leq 0.05$ will be used for all statistical comparisons. The optimal treatment regimen (concentration and exposure time) will be the treatment that produces the lowest percent mortality. A treatment will be determined not to be efficacious if the treatment does not result in a statistically significant reduction in fish mortalities.

Identification of Bacteria. Prior to initiation of all treatments, personnel will identify and document the presence of external pathogenic bacteria (*Flavobacters*) on individual fish by methods described by Warren (1981), Post (1987) or Lasee (1995). Three replicates of four fish will be removed from the composite fish sample for disease identification. Personnel conducting the studies will be trained in the identification of fish diseases. Also, to assure accurate identification of the disease, heatfixed slide preparations of the sampled tissues (Lasee 1995) will be prepared at the study site and archived in case later confirmatory tests are deemed necessary.

Tissue residue depletion data for florfenicol, oxytetracycline, and chloramine-T (Objective 2)

Florfenicol and Oxytetracycline

This study will be conducted with walleye at two locations. The fish acclimation, treatment, depuration, and dissections will be conducted at the IDNR RFCRF co-located at 15053 Hatchery Place, Moravia, Iowa 52571-8933. The feed and fillet tissue analysis for the drugs will be conducted at the UMESC located at 2630 Fanta Reed Road, La Crosse, Wisconsin 54603.

Fiberglass rectangular tanks (3.35 m long × 0.76 m wide) located inside the RFCRF building and supplied with flowing water from the Rathbun Reservoir will be the exposure systems. The treatment rectangular tanks will contain approximately 1550 L of water. The water flow rate through the exposure tanks will be maintained at greater than 26 L/min. The flow rate will be monitored following the hatchery's standard procedures (calibrated bucket and stop watch). The fish will be subjected to lighting by metal halide lamps during the day and low intensity fluorescent lights at night inside the building. Photoperiod data and light intensity data will be collected and stored in the data management system for UMESC. Personnel

assigned to the study will record any maintenance performed on the exposure system during the acclimation treatment and depuration periods.

During the acclimation, treatment, and depuration periods of the study, the water temperature in the treatment system will be approximately 18 ± 2°C. The water quality is expected to remain within the historical limits of the hatchery. Ranges of pH and dissolved oxygen are expected to fluctuate with temperature, but should remain within the following limits: pH, 7.0-8.5; dissolved oxygen, greater than 5.0 mg/L and 60% saturation. Temperature, pH, dissolved oxygen, and saturation will be monitored at least hourly throughout the treatment and depuration periods with YSI Model 600 water quality monitoring probes. Ammonia concentrations will not be monitored unless the water flow rate drops below one tank exchange per hour. The concentration of the drugs in the water will not be determined.

Fish care and maintenance procedures will follow standard walleye rearing practices at RFCRF. These procedures are similar to UMESC animal care and maintenance guidelines. Stocking densities and water flow rates will be within the requirements as determined under consultation with the RFCRF Natural Resource Biologist. The treatment tank will be cleaned daily following the normal RFCRF cleaning procedures.

To assure medicated feed will be eaten, fish will be allowed to acclimate in the treatment system until they are feeding well as judged by a trained/experienced RFCRF fish culturist. Normal feeding methods at RFCRF will be followed. The fish will be fed using automatic feeders that dispense pellets every 10 min 24 hr per day. The feeders will be replenished daily. The same ratio of feed to total fish mass (4%) will be offered to all fish during the acclimation, treatment, and depuration periods of the study. During the 10-day treatment period, there will be no attempt to increase the feed mass offered the fish to account for fish growth.

Walleye Grower 9206, 3.0 mm from Nelson & Sons Inc., Murray, Utah, will be fed to the fish throughout the study. Non-medicated Walleye Grower 9206 coated with cod liver oil (1 mL oil/20 g feed) will be offered during the acclimation period; florfenicol or oxytetracycline medicated Walleye Grower 9206 will be offered during the treatment period, and non-medicated Walleye Grower 9206 feed without cod liver oil amendment will be offered after the treatment period.

Fish will be acclimated to feeding the oil-coated non-medicated feed for at least three days before treatment. Fifty fish will be sampled and filleted at least two days before treatment. Paired fillets will be used for analytical method verification, storage stability tests, and analytical control tissue. The remaining fish will be offered medicated feed during the treatment period. On designated treatment days, 20 fish/day will be collected for examination of drug concentration in the fillet tissue. The first day of depuration will start immediately after the last day of treatment, when the fish will be offered non-medicated feed. The automatic feeder will be thoroughly cleaned (soap and water followed by methanol) before feeding the non-medicated feed. Twenty test fish per day will be collected and filleted on depuration days 1, 2, 3, 4, 7, 9, and 14. The drug base concentration in the fillet tissues from the sampling periods on depuration days 1, 2, and 3 will be determined before depuration day 14. Pending results of the apparent drug depletion from the initial three depuration periods, up to three more sample sets of 20 fish each may be collected. The number of sample sets (1, 2, or 3 sets) and the dates)/time these additional sample sets would be collected depend on the results of the initial three depuration sampling periods and will be decided on in consultation with UMESC pharmacologist after the initial data is collected.

Three types of supporting data will be collected to establish that the fish were feeding during the treatment and depuration phases of the study. First, the presence of fecal casts in the tank will be monitored daily. Second, the stomach and intestines will be examined for presence of feed during sampling. Third, the growth of the fish (length and weight data of sacrificed fish at each sample period) will be plotted for trend analysis.

Chloramine -T

During bath treatment residue testing with chloramine-T, fish will be fed an appropriate nonmedicated diet at 4% of total fish mass in the exposure tank. Test fish will be exposed to the maximum concentration and duration allowed by the proposed label, then followed through the depuration period.

For chloramine-T testing, the exposure system, water quality data, and fish culture procedures are similar to those explained in the feeding studies. Fish will be acclimated to the tank system at least two days before exposure begins. The first day of depuration will start immediately after the last day of treatment.

Two to three samples consisting of 20 fish will be taken during the treatment period and additional samples will be taken on depuration days 1, 2, 3, 4, 7, 9, and 14. Once again, depending on the initial results from the first three depuration samples, additional samples may be required beyond 14 days.

Fish Sampling. At each sampling, a group of fish in the tank will be captured with a dip net and a maximum of five fish will be removed indiscriminately from the net and transferred to the electronarcosis unit. The remaining fish will be returned to the tank. All fish in the electronarcosis unit will be sacrificed simultaneously. Each euthanized fish will be rinsed in fresh flowing water, and placed in a plastic bag on ice. Groups of fish will continue to be captured from the tank until all fish for that sampling period (50 fish for controls during acclimation and 20 fish for all treatment and depuration sampling periods) have been taken. The location in the tank will change for each group of captured fish so that fish are taken from all segments of the tank. Data from these procedures will be recorded and will be placed in the data management system for UMESC. The fish will be alive in the electronarcosis unit for less than two minutes. The volume of water in the electronarcosis unit will be measured and the average volume versus biomass of fish ratio will be calculated. The water volume to biomass ratio and the oxygen content of the water in the electonarcosis unit will be large enough to ensure that the fish will not be stressed before electrocution.

Data Reporting And Statistical Methods. In this study, fish tissue analysis results will be expressed as concentrations of drug base and feed analysis results will be expressed as concentrations of oxytetracycline-HCl or florfenicol.

Statistical analysis of the florfenicol and oxytetracycline concentration in feed samples will be limited to simple expressions of means, standard deviations (SD), relative standard deviations (RSD), and analysis of variance.

Statistical analysis for chloramine-T, florfenicol, and oxytetracycline concentration data on replicate analyses of individual fish's fillet tissue will be limited to simple expressions of means, SD, RSD, and confidence intervals. The drug concentration in the fillet tissue at each sampling time and the drug depletion rate will be analyzed using a regression analysis.

FACILITIES

The RFCRF is a 372 m^2 facility that contains a water processing plant, office/laboratory, tank room, and climatized feed storage room. Inside the tank room are 15 fiberglass raceways, 9 circular tanks, and an egg incubator. Twelve concrete raceways are located outside the building. The facility is dedicated to fish culture research and disease diagnosis.

The Spirit Lake Hatchery may also be used for any of the previously described studies; however, this facility will primarily work with walleye. The hatchery is a coolwater hatchery with 12 indoor rearing tanks and 4 egg hatching batteries. The facility produces walleye and muskellunge for the State of Iowa. It will be used as a back up if RFCRF cannot generate the required disease epizootic.

REFERENCES

Lasee, B.A. 1995. Introduction to fish health management, second edition. Fish and Wildlife Publication.

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler, and J.R. Leonard.1982. Fish hatchery management. U.S. Department of the Interior Fish and Wildlife Service, Washington, D.C.

Post, G.W. 1987. Textbook of fish health. TFH Publications, Inc., Ltd., Neptune City, New Jersey.

Warren, J.W. 1981. Diseases of hatchery fish. U.S. Department of Interior Fish and Wildlife Service, Twin Cities, Minnesota.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Iowa Department of Natural Resources 15053 Hatchery Place Moravia, IA 52571				**USDA AWARD NO.** Objectives 1-2	
				Duration Proposed Months: 12	Duration Awarded Months:
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Alan A. Moore				**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. 1 (Co)-PI(s)/PD(s)	1.6			$6,263	
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$2,087	
Total Salaries and Wages →				$8,350	
B. Fringe Benefits (If charged as Direct Costs)				$0	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$8,350	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$7,465	
F. Travel 1. Domestic (Including Canada)					
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Utilities ($800)				$800	
J. Total Direct Costs (C through I) →				$16,615	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$16,615	
M. Other →					
N. Total Amount of This Request →				$16,615	$
O. Cost Sharing (If Required Provide Details)	$				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR IDNR

Objectives 1 and 2

A. Salaries and Wages. 1.6 months of the PI's time will be required to complete the objectives; the assistance of a fisheries aide for 284 hours (@ $7.35/hour) will also be required.

E. Materials and Supplies. Fish feed ($825) and fish ($6,640).

I. Other Direct Costs. Electrical cost of heating water for approximately two months of the study ($800).

PRINCIPAL INVESTIGATOR

Allan A. Moore, Iowa Department of Natural Resources

LIST OF ADDITIONAL INVESTIGATORS

Jeff Bernardy, Upper Midwest Environmental Sciences Center

Jeff Rach, Upper Midwest Environmental Sciences Center

Guy Stehly, Upper Midwest Environmental Sciences Center

ATTACHMENT

VITA

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EDUCATION

B.S. Iowa State University, 1969

POSITIONS

Fish Hatchery Research Biologist (1981-present), Iowa Department of Natural Resources
Manager (1979-1981), Rathbun Fish Hatchery, Iowa Dept of Natural Resources
Assistant Manager (1975-1979), Rathbun Fish Hatchery, Iowa Conservation Commission
Assistant Manager (1969-1975), Blind Pony Fish Hatchery, Missouri Dept of Conservation

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Iowa Chapter, American Fisheries Society
Iowa Aquaculture Association
Working Group on Quality Assurance in Aquaculture Production

SELECTED PUBLICATIONS

Moore, A.A., M.A. Prange, B.T. Bristow, and R.C. Summerfelt. 1994. Influence of stocking density on walleye fry viability in experimental production tanks. Progressive Fish Culturist 56(3):194-201.

Moore, A.A., M.A. Prange, R.C. Summerfelt, and R.P. Bushman. 1994. Evaluation of tank shape and use of surface spray for rearing larval walleye on formulated feed. Progressive Fish Culturist 56(2):100-110.

Moore, A.A., M. Mason, and J. Morris. 1992. Managing Iowa's Fisheries; Getting started in aquaculture enterprises. Iowa State University Press, Ames, Iowa.

Moore, A.A. 1992. Passive Integrated Transponder tagging of channel catfish. Progressive Fish Culturist 54(2):125-127.

Ringle, J.P., J.G. Nickum, and A.A. Moore. 1992. Chemical separation of channel catfish egg masses. Progressive Fish Culturist 52(2):73-80.

Moore, A.A., M.E. Eimers, and M.A. Cardella. 1990. Attempts to control Flexibacter columnaris epizootics in pond reared channel catfish by vaccination. Journal of Aquatic Animal Health 2(2):109-111.

Moore, A.A. 1987. Short term storage and cryopreservation of walleye semen. Progressive Fish Culturist 49(1):40-43.