STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR CHLORAMINE-T (INAD #9321)

Sponsor:

U.S. Fish and Wildlife Service, Fisheries and Habitat Conservation

_____ _____

Sponsor Signature Date Approved

Manufacturers:

Axcentive SARL
525 West Van Buren Street
Chicago, IL 60607-3835

B.L. Mitchell, Inc.
1774 E. Azalea Dr.
Greenville, MS 38701-7505

Facility for Coordination of Chloramine-T INAD:

USFWS's Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date:	September 1, 2007
Proposed Ending Date:	August 31, 2012
Study Director:	Mr. Jim Bowker

_____ _____

Study Director Signature Date

Clinical Field Trial Location and Trial Number:

Facility _____

Type or Print Name

Investigator _____

Type or Print Name

_____ _____

Investigator Signature Date

STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR CHLORAMINE-T UNDER INAD #9321

I. STUDY ID AND TITLE:

Clinical field trials to determine the efficacy and safety of Chloramine-T immersion therapy to control mortality caused by bacterial gill disease and external flavobacteriosis in a variety of freshwater fish species.

II. SPONSOR:

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

Manufacturers: Axcentive SARL
525 West Van Buren Street
Chicago, IL 60607-3835

Contact: Larry Holzman (International Specialty Chemicals, Inc.)
Phone: 914-333-0606
Fax: 914-333-0333
Email: lbh@ischem.com

B.L. Mitchell, Inc.
1774 E. Azalea Dr.
Greenville, MS 38701-7505

Contact: Betty Mitchell
Phone: 662-686-9002
Fax: 662-686-9020
Email: blmitch@bellsouth.net

Study Director: Mr. Jim Bowker, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9910; Fax: 406-582-0242; Email: jim_bowker@fws.gov

Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

Study Monitors: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES:

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Reauthorization Starting Date: September 1, 2007

Reauthorization Expiration Date: August 31, 2012

Proposed Termination Date: To be determined by research progress.

V. BACKGROUND/PURPOSE:

A. Bacterial Gill Disease - Bacterial gill disease (BGD) is a potentially acute disease of intensively cultured fish, particularly young salmonids. If it is not diagnosed and treated early, epizootics may occur within a 24 hour period. Affected fish stop feeding, swim near the surface, and orient themselves into the current to optimize oxygen absorption. Microscopically, gill epithelium is hyperplastic and covered with masses of long, thin gram-negative bacteria. Although death is caused by the massive, smothering infection of the gills, stressors associated with intensive culture, such as crowding and low concentrations of dissolved oxygen, often predispose fish to infection. Neither the stressors involved nor their modes of action are fully understood. Although flavobacteria may be common, no single pathogen appears to be responsible for all cases of bacterial gill disease. Known agents are gram-negative bacteria and include, in addition to flavobacteria, flexibacteria, aeromonads, and pseudomonads (Snieszko 1981; Post 1987). Moreover, gill lesions infected with these organisms provide excellent habitat for opportunistic fungi (Warren 1991).

B. Flavobacteriosis - Flavobacteriosis, caused by *Flavobacterium columnare,* Flavobacterium phychrophilum, or closely related yellow pigmented gliding bacteria as described in U. S. Food and Drug Administration (FDA) Public Master File #5456, is an acute to chronic bacterial infection that has been reported as a mortality factor in many species of cultured salmonids, catfish, bait minnows, goldfish, basses, and sunfish. Severe epizootics occur in both natural and cultured fish populations at a wide range of water temperatures. Although highly virulent strains of flexibacters can cause disease outbreaks even under good environmental conditions, stressors such as oxygen depletion, accumulation of waste products and un-eaten food, crowding and handling, or species susceptibility are common predisposing factors.

The transmission of flavobacteriosis from fish to fish occurs directly through the water. The bacteria invade through breaks in the skin. The type of lesion varies with the species of fish. In scaleless fish such as channel catfish, the lesions are small and circular with gray-blue necrotic centers and red margins surrounded by a ring of inflamed tissue. In scaled fish, such as salmonids, necrotic lesions begin at the outer margin of the fins and spread toward the body. When the gills are infected they may show light-colored areas at the tips of the filaments. As the disease progresses, the gill filaments become eroded and soft-tissue sloughing is common (Bullock et al. 1986). As the disease progresses, flexibacters may invade the blood stream through a gill or skin lesion resulting in systemic infections. Careful diagnostic work is required to distinguish between BGD and flavobacteriosis infections of the gills (Warren 1991).

C. Control of BGD and Flavobacteriosis - Chloramine-T is effective in controlling BGD in cultured fishes (From 1980; Bullock et al. 1991). Salmonids are relatively tolerant of the chemical. Bills et al. (1988) established the 1 hour LC_{50} value of Chloramine-T to rainbow trout as greater than 60 mg/L except in soft acidic (pH 6.5) waters where the 1 hour LC_{50} was 55.8 mg/L. Bullock et al. (1991) recommend a bath treatment of 8.5 mg/L of Chloramine-T for 1 hour as an effective treatment for BGD in cultured salmonids; however, results are best in clear, clean water and when treatments are begun in the early stages of an outbreak. Bullock et al. (1991) state that a second or third treatment may be required if an outbreak goes untreated until it is in an advanced stage or the fish have been under stress for a prolonged period. The chlorine demand of silty water or water with a high organic load may necessitate dosages as high as 20 mg/L or repeated treatments for adequate disease control.

Chloramine-T is also considered to be effective in controlling early, external flavobacteriosis in cultured fishes, based on observations by staff at the National Fish Health Laboratory (Leetown, WV) and anecdotal observations by hatchery managers throughout the United States. They consider the treatment of fish with 8.5 mg/L of Chloramine-T for 1 hour, in a flow-through or standing bath treatment, adequate to control external flavobacteriosis.

Integrated fish health management practices are used to prevent the occurrence of these diseases. However, adverse environmental conditions, physiological changes related to stressors, uncontrollable water conditions, and unforeseen factors can lead to severe disease outbreaks requiring prompt treatment to prevent significant losses in excess of 50 percent of fish in public, tribal and private aquaculture. Such treatment also reduces the discharge of infectious agents into the natural environment thereby reducing the potential spread of disease.

Treatment strategies for the use of Chloramine-T in fish shall be designed to meet the needs of each species or lot, the size and numbers of fish to be treated, the layout of the facility, and environmental conditions. In all cases the objective shall be to minimize the impacts of disease on fish health, fish quality and survival, and to fully meet fishery management or aquaculture objectives. Because there are many factors that can affect the success or failure of Chloramine-T immersion therapy, data are needed to determine the best methods to use this drug to obtain effective disease control. Complete documentation of studies that are well conceived and well carried out will be of great value.

The primary purpose of this Investigational New Animal Drug (INAD) exemption is to obtain additional clinical field trial data to demonstrate the efficacy and target animal safety of Chloramine-T immersion therapy to control mortality caused by BGD and external flavobacteriosis in a variety of freshwater fish species under a variety of environmental conditions. Efficacy trials will be conducted at a number of different study sites, on a variety of fish species infected with several of fish pathogens loosely grouped within the BGD or external columnaris categories.

The U.S. Fish and Wildlife Service (USFWS) anticipates that it may require several years to carry out all clinical field trials and laboratory studies required to obtain efficacy and safety data required to support the specific label claims necessary to cover major aquaculture needs. Therefore, the USFWS may request that the U. S. Food and Drug Administration (FDA) grant re-authorization of this Chloramine-T INAD sometime in the future. In the interim, the USFWS will continue to work closely with the sponsor, the National Coordinator for Aquaculture New Animal Drug Applications, and other research and conservation agencies to develop other required New Animal Drug Application (NADA) research data to support labels

claims for Chloramine-T. Therefore, clinical field trials planned under this particular INAD are but one part of a larger coordinated and diligent inter-agency effort that will eventually meet all Chloramine-T NADA data requirements.

VI. SPECIFIC OBJECTIVES:

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the effectiveness and safety of Chloramine-T immersion therapy to control mortality caused by BGD and external flavobacteriosis in a variety of freshwater fish species.

2. Provide an opportunity for fish culturists to legally use Chloramine-T immersion therapy to control mortality caused by BGD and external flavobacteriosis in a variety of freshwater fish species so that they can maintain healthy stocks of fish during the period of time necessary for collection of data that will be used to support a NADA(s) for Chloramine-T.

Specific study objectives of this study protocol are as follows:

Objective A

To determine if bacterial gill disease or external flavobacteriosis can be prevented by treating fish with a single 15 mg/L dosage of Chloramine-T, for one hour duration, administered one day per week. This series of weekly treatments may be started and ended, as recommended by the fish health biologist on a schedule based upon the disease history of the facility for the fish species involved. **Note:** Disease prevention studies may be conducted on salmonids, sturgeon, perch, sunfish, bass and other coolwater and warmwater fish species listed in Appendix VIa only if the cause of death of fish in all rearing units is closely monitored throughout the trial, and a portion of the experimental units are maintained as untreated controls or a separate controlled study is established elsewhere on the facility. If outbreaks of bacterial gill disease or external flavobacteriosis occur in the control units or if disease prevention fails, one of the disease treatment regimes set forth in Objectives B, C, or D may be used to prevent excessive losses of fish.

Objective B

To determine the efficacy of three consecutive or alternate daily bath (standing bath or flow-through) treatments of 10, 15, or 20 mg/L of Chloramine-T, for one hour duration, **for the treatment of bacterial gill disease in a variety of salmonid fish species** listed in Appendix VIa when treated in a variety of rearing or environmental conditions.

Objective C

To determine the efficacy of three consecutive or alternate daily bath (standing bath or flow-through) treatments of 10, 15, or 20 mg/L of Chloramine-T, for one hour duration, **for the treatment of external flavobacteriosis in a variety of salmonid fish species** listed in Appendix VIa when treated in a variety of rearing or environmental conditions.

Objective D

To determine the efficacy of three consecutive or alternate daily bath (standing bath or flow-through) treatments of 10, 15, or 20 mg/L of Chloramine-T, for one hour duration, **for the treatment of BGD and external flavobacteriosis in sturgeon, perch, sunfish, bass, and other coolwater and warmwater fish species** listed in Appendix VIa when treated in a variety of rearing or environmental conditions.

VII. MATERIALS:

A. Test and Control Articles:

1. Drug Identity

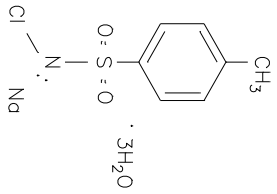
a. Active ingredient

Common Name: Chloramine-T

Product Name:: **Halamid**® (Axcentive SARL)
Actamide (B.L. Mitchell, Inc.)

Chemical Description: Sodium p-toluenesulfonchloramide (**Halamid**®)
n-chloro-para-toluene sulfonamide sodium salt (**Actamide**)

CAS Number: 7080-50-4 (**Halamid**®)
127-65-1 (**Actamide**)



Appearance: White crystalline powder

Odor: Weak chlorine odor

b. Strength and dosage form

Chloramine-T, as used for treating BGD or external flavobacteriosis in fish, is a water soluble compound and is not formulated in any way. For treatment calculation purposes, Chloramine-T contains 100% active ingredient.

c. Manufacturer(s), source of supply

Axcentive SARL
525 West Van Buren Street
Chicago, IL 60607-3835

Contact: Larry Holzman (International Specialty Chemicals, Inc.)
Phone: 914-333-0606
Fax: 914-333-0333
Email: lbh@ischem.com

B.L. Mitchell, Inc.
1774 E. Azalea Dr.
Greenville, MS 38701-7505

Contact: Betty Mitchell
Phone: 662-686-9002
Fax: 662-686-9020
Email: blmitch@bellsouth.net

2. Verification of drug integrity/strength:

The Manufacturers will provide the analytical data necessary to establish the purity of each lot of Chloramine-T supplied. The lot number and date of manufacture for each batch of Chloramine-T will be placed on the label of each container. The form "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (Form CLT-1) will clearly identify the lot number and date of manufacture of all Chloramine-T shipments. If the integrity of the Chloramine-T is compromised (i.e., by spilling or contamination of the stock container) the Chloramine-T must not be used for treatment, and the event should be carefully recorded, dated, and signed in the Chemical Use Log (Form CLT-2). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

Chloramine-T must be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. The container should be stored out of direct sunlight in a dry, well ventilated area at room temperature. *Do not refrigerate*. The storage unit for Chloramine-T must be labeled to indicate that it contains hazardous material and that "*NO Food or Drink is to be Stored in this unit*". Chloramine-T should be stored in a secure location such as in a locked cabinet.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for Chloramine-T (see Appendix IV). Each person involved with the study and each person who may be present during the use of Chloramine-T shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with Chloramine-T.

5. Investigational labeling

Copies of the labels to be attached to each container of Chloramine-T are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of Chloramine-T.

6. Accountability

Axcentive SARL and B.L. Mitchell, Inc. will be the sole suppliers of Chloramine-T to all Investigators under INAD 9321.

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of Chloramine-T, the Investigator will complete Form CLT-1 “Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The investigator will archive the original in the facilities INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form CLT-1. The Study Monitor will then forward a copy to the Study Director at the AADAP Office. The Study Director will archive one copy, and send two copies of Form CLT-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form CLT-1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of Chloramine-T on-hand. A Chemical Use Log (Form CLT-2) will be supplied to each Investigator. Each time Chloramine-T is used, it must be recorded by the Investigator on Form CLT-2.

7. Preparation Procedures

Chloramine-T will be supplied to Investigators as a 100% active ingredient drug. Prior to actual use for treatment, a calculated and weighed amount of Chloramine-T (based on a pre-determined target treatment concentration of 10, 15, or 20 mg/L) should first be dissolved in a small volume of ambient temperature rearing water to establish a stock solution. After thorough mixing of chloramine-T, the stock solution should then be applied to, and thoroughly mixed with, rearing unit water. Chloramine-T should not be adulterated in any manner prior to use. Following completion of treatment, Chloramine-T should be flushed from the rearing unit.

B. Items Needed for Treatment, Data Collection, Etc.:

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

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by Chloramine-T INAD 9321 will need to complete several forms. These forms are described in Section XIII (p. 13). Copies of these forms are attached to this Study Protocol.

VIII. EXPERIMENTAL UNIT

The experimental unit in these clinical field trials will consist of contained or isolated groups of fish. This will generally be a groups of fish contained in tanks, raceways, or ponds. However, the experimental unit in clinical field trials may also be **individual animals**. If individual animals are considered to be the experimental unit, treatment response parameters for each animal must be evaluated separately.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol before Chloramine-T can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (See Section XX).

B. The characteristics of the study animals (species, number, etc.) is presented in Appendix VIa and Appendix VIb.

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on a Form CLT-3a or Form CLT-3b.

D. Ability of Investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

E. Pathogen/disease considerations

A. Bacterial fish pathogens should be presumptively identified by procedures described in Section 1 of the Fish Health Section Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. 2005 Edition, Fish Health Section/American Fisheries Society. Other, more sensitive methods described elsewhere in peer-reviewed references, or as mutually determined by the local fish health biologist, in consultation with the Study Monitor, also may be used. (**Note: Diagnostic methods other than those in the 2005 Edition of the "Blue Book" should be described on a separate sheet attached to a Form CLT-3a or Form CLT-3b "Results Report Forms"**).

B. Typically, there should be increased mortality rates among fish in a rearing unit(s) for three or more consecutive days. (Note: Station history and the experience of the investigator, monitor, or the fish health biologist may over-ride this criterion to halt potentially explosive disease outbreaks. In such cases, however, careful diagnostic surveillance should be carried out in all rearing units proposed for treatment and controlled tests should be carried out if at all possible).

C. Typical disease signs should be detectable in at least a few fish and the causative bacterial pathogen must be presumptively identified.

D. Since the efficacy of Chloramine-T immersion therapy for the control of mortality caused by BGD and external flavobacteriosis is being tested, investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other treatments once a decision has been made to conduct Chloramine-T therapy. Complicating bacterial or other aquatic

pathogens should be carefully documented. If necessary, these infections can be treated once Chloramine-T response (efficacy) data has been collected. However, it may require as long as 10 days after the completion of Chloramine-T immersion therapy to determine differences between test and control groups and to complete post-treatment evaluations.

Prior to initiating each treatment event, the Investigator must first complete Form CLT-W. "Worksheet for Designing Individual Field Trials" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent by Fax to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward (Fax) the paperwork to the AADAP Office. The AADAP Office will then review the worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on Form CLT-2, Form CLT-3a, and/or Form CLT-3b, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, and infection/disease/treatment need is rapidly escalating, the Investigator should contact the AADAP Office for a study number and permission to proceed.

X. TREATMENT GROUPS

A. A treatment group or experimental unit may be an entire tank, pond, raceway, group of fish, or it may be individual animals.

B. Separately confined, untreated control fish <u>will not</u> be required in supplementary field studies conducted to determine the effectiveness and safety of Chloramine-T immersion therapy. Fish from a group or lot will first be examined to determine if treatment with Chloramine-T is required. When treatment is underway or has been completed, fish from the same group will be examined to determine the effect of treatment on the parameters used to initially sanction the treatment. Evaluation will in all cases consist of determining fish mortality, although in some cases degree or severity of bacterial infestation may also be quantified.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are <u>strongly encouraged</u> whenever circumstances permit. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals. Use of control groups will ensure that results of efficacy studies provide useful information that will support an NADA.

It is important that all fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Although not required, replicate treatment groups are strongly encouraged in both treated and control groups. Assignment to control and treatment groups should be random and designed to avoid bias.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which treatment response observations are recorded by individuals who are unaware which fish have been treated and which fish are controls.

The designation of specific treatment groups often depends upon the number of affected treatment units, the nature and severity of the disease being treated, and the variables being tested. Two general categories of treatment groups are generally anticipated.

1. **Spotty, low level, or chronic disease patterns:**

A number of facilities participating in this INAD are doing so as a means of being prepared, in advance, to use Chloramine-T in the event a bacterial disease outbreak occurs. If management practices have been good, disease occurrences often result in low morbidity and mortality rates. Chloramine-T immersion therapy may be occasionally required as a part of a comprehensive fish health management program. These situations are the most typical. Even though there may be too few units involved to allow for treatment replication, careful record keeping is important so that useful data can be collected. Handling of clinically ill fish populations should be kept to a minimum until they have been successfully treated. Even the careful separation of diseased fish into new groups for treatment may alter environmental conditions present during disease initiation, thereby potentially rendering the Chloramine-T immersion therapy trial meaningless.

2. **Epizootics:**

At some participating facilities disease outbreaks may be more widespread, more severe, and occur more regularly. Sufficient fish and test units at these facilities may be available to conduct higher quality studies (i.e. studies that include replication, randomization, blinding, etc.). Such situations are suitable for the conduct of pivotal, carefully designed, and controlled studies. Investigators at these facilities are encouraged to contact the AADAP Office for assistance with study design and completion. These facilities will be given top-priority for the availability of guidance, on-site assistance from the AADAP Office, and diagnostic support from fish health biologists.

XI. TREATMENT SCHEDULES

A. Route of administration

Chloramine-Twill be administered only as an immersion treatment. Investigators may use either a static-bath or flow-through treatment regimen.

B. Dosage and treatment duration

Objective A [For the prevention of BGD and external flavobacterisis in salmonids, sturgeon, perch, sunfish, bass and other coolwater and warmwater fish species]

Chloramine-T should be administered at a treatment dosage of 15 mg/L.

Treatment duration is 1 hour.

Objective B [For the treatment of BGD in salmonid fish species]

Chloramine-T may be administered at treatment dosages of 10, 15, or 20 mg/L.

Treatment duration is 1 hour.

Objective C [For the treatment of external flavobacteriosis in salmonid fish species]

Chloramine-T may be administered at treatment dosages of 10, 15, or 20 mg/L.

Treatment duration is 1 hour.

Objective D [For the treatment of BGD and external flavobacteriosis in sturgeon, perch, sunfish, bass, and other coolwater and warmwater fish species]

Chloramine-T may be administered at treatment dosages of 10, 15, or 20 mg/L.

Treatment duration is 1 hour.

C. Dosing interval and repetition

Chloramine-T will be administered as a single treatment regime, with no repetition of treatment.

D. Drug preparation and administration procedures

Chloramine-T will be supplied to Investigators as a 100% active ingredient drug. Prior to actual use for treatment, a calculated and weighed amount of Chloramine-T (based on a pre-determined target treatment concentration of 10, 15, or 20 mg/L) should first be dissolved in a small volume of ambient temperature rearing water to establish a stock solution. After thorough mixing of chloramine-T, the stock solution should then be applied to, and thoroughly mixed with, rearing unit water. Chloramine-T should not be adulterated in any manner prior to use. Following completion of treatment, Chloramine-T should be flushed from the rearing unit.

E. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct Chloramine-T immersion treatment. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the Chloramine-T immersion treatment involved should be appropriately labeled.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or other pertinent species information indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form CLT-3 and/or Form CLT3b. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

   Morbidity and mortality data, coupled with case history and analyses of bacterial load, usually indicate when Chloramine-T immersion treatment is needed. **Typically, source data must be collected for at least 5 days before treatment, during treatment, and for up to at least 10 days after the treatment period has ended**. Collection of this data is critically important. Samples of gill, skin, fin, or mucous may also be removed from groups of representative fish and microscopically evaluated to determine the presence (or absence) of target pathogens.

   The only exception to the requirement for the collection of morbidity and mortality data is with respect to the treatment of all salmonid species for the control of mortality caused by BGD (Objective B). These data are not required as the NADA efficacy technical sections to support this claim has already been accepted by CVM.

2. Secondary Parameters

   Secondary parameters may also include general observations on fish behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

3. Adverse Reactions

   Any adverse reaction to treatment should be reported immediately to the Study Monitor, who will in turn notify the Study Director. Such responses might include extremely negative responses/behavior by the fish or hazards to the applicator. Although Chloramine-T immersion therapy has been used extensively for many years with beneficial effect in fish culture, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Investigators should carefully observe all treated fish for <u>any</u> signs of adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions. If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.

   Note: Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by Chloramine-T INAD 9321 will need to complete the following forms:

Form CLT-W. Worksheet for Designing Individual Field Trials under Chloramine-T INAD 9321

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

Form CLT-2. Chemical Use Log for Clinical Field Trials under Chloramine-T INAD 9321

Form CLT-3a. Results Report Form for use of Chloramine-T under INAD 9321 - All samonids treated for BGD at 12 - 20 mg/L; treatment on 3 consecutive or 3 alternate days

Form CLT-3b Results Report Form for use of Chloramine-T under INAD 9321 - All use excluding samonids treated for BGD at 12 - 20 mg/L; treatment on 3 consecutive or 3 alternate days

Copies of these forms are attached to this Study Protocol.

XIV. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data should be original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it. Each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes. The original record should be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. All fish treated with Chloramine-T immersion therapy may be released immediately following the completion of treatment (i.e., the withdrawal time is 0-days). The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form CLT-3a and/or Form CLT-3b. Also, note that the Investigator is also requested to estimate the predicted number of days/months before treated fish will be susceptible to harvest and/or human consumption on Form CLT-3a and/or Form CLT-3b.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Chloramine-T will be used only in the manner and by the individuals specified in the Study Protocol. If any unused Chloramine-T remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Typically, compromised or out-dated Chloramine-T should be disposed of in a landfill. Although excess (and un-compromised or out-dated) Chloramine-T may be redistributed to other facilities listed in the Study Protocol, Chloramine-T may not be redistributed to other facilities not specified in the Study Protocol.

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

A. Drug distribution

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

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor should be assigned to each facility that is authorized to treat fish with Chloramine-T immersion therapy. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the Chloramine-T itself) are typically readily available at each participating fish hatchery. The use of various drugs, chemicals, and therapeutants to meet management and/or production goals is a common occurrence at most fish hatcheries. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 7).

D. Administrator of the drug

Chloramine-T will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Chloramine-T will be maintained in a secure location, and only the Investigator or persons under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 6) for details and Forms CLT-W, CLT-1, CLT-2, CLT-3a, and CLT-3b (page 13) for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitor who will review the information and ensure that all required data are provided. The Study Monitor will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare an annual report that will be submitted to the FDA.

G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form CLT-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. A copy of Form CLT-2 should be sent to the Study Monitor with the corresponding Form CLT-3a (and/or Form CLT-3b). A copy of Form CLT-3a and/or Form CLT-3b should be sent to the Study Monitor after completion of the entire treatment period, which includes the post-treatment observation period. **All forms must be submitted by the end of the calendar year**. The Study Monitor should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, the Study Monitor should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared and submitted to the FDA. This submission will probably include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

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

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation**. Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with Form CLT-3a and/or Form CLT-3b, and ultimately be submitted to the Study Director.

LITERATURE CITED

American Fisheries Society. 2005. Fish Health Section Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. 2005 Edition, Fish Health Section/American Fisheries Society, Bethesda, MD

Bills, T. D., L. L. Marking, V. K. Dawson, and J. J. Rach. 1988. Effects of environmental factors on the toxicity of chloramine-T to fish. U.S. Fish and Wildlife Service, Investigations in Fish Control No. 96. 6 pp.

Bullock, G. L., R. L. Herman, and C. Waggy. 1991. Hatchery efficacy trials with chloramine-T for control of bacterial gill disease. Journal of Aquatic Animal Health 3:48-50.

Bullock, G. L., T. C. Hsu, and E. B. Shotts, Jr. 1986. Columnaris disease of fishes. U.S. Fish and Wildlife Service, Fish Diseases Leaflet 72. 9 pp.

From, J. 1980. Chloramine-T for control of bacterial gill disease. The Progressive Fish-Culturist 42(2):85-86.

Post, G. W. 1987. Textbook of fish health. Revised and expanded edition. TFH Publications, Inc., Ltd., Neptune City, New Jersey. 288 pp.

Snieszko, S. F. 1981. Bacterial gill disease of freshwater fishes. U.S. Fish and Wildlife Service, Fish Disease Leaflet 62. 11 pp.

Warren, J. W. 1981. Diseases of hatchery fish. U. S. Fish and Wildlife Service, Twin Cities, Minnesota. 91 pp.

Appendix IV. Safety Data Sheet (SDS) for Halamid® Aqua

The SDS for Halamid® Aqua can be found at the drug sponsors website

http://www.syndel.com/downloads/dl/file/id/95/halamid_aqua_sds.pdf

Chloramine-T Clinical Field Trials
CLT-W: Worksheet for Designing Study Numbers - Version 4
Chloramine-T INAD 9321

INSTRUCTIONS

1. Investigator must fill out Form CLT-W for each trial conducted under this INAD **before** actual use of Chloramine-T. The Investigator is responsible that Form CLT-W is completed accurately.
2. Investigator should keep the original on file, and Fax a copy to the Study Monitor for review.
3. After review, the Study Monitor will fax a copy to the Bozeman NIO for assignment of the Study Number.
4. The Bozeman NIO will review the worksheet, and then fax the assigned trial Study Number to both the Investigator and Study Monitor, at which time the trial may be initiated.
5. **Note:** Both Investigator and Study Monitor should sign and date Form CLT-W.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species to be treated		Disease to be treated	
Average fish weight (gm)		Average fish length (in)	
No. of fish per unit (e.g. 10,000 fish/raceway)			
Number of treated units		Number of treated fish	
Number of untreated control units		Number of control fish	
Anticipated date treatment will be initiated			
Check type of treatment method used		_____ Flow through bath	_____ Standing
Check type of treatment		_____ Disease control prevention	_____ Disease
Intended drug target dosage (mg/L)	10 mg/L 15 mg/L 20 mg/L	Estimated total amount of drug needed for proposed treatment (Kg)	
Drug manufacturer		Drug lot number	

STUDY DESIGN: Describe in detail the purpose of the clinical trial. For example you might compare dosage, treatment frequency, or treatment method (Flow-Through vs. Standing Bath). Study design must be carefully focused and lend itself to rigorous evaluation. If more space is required to describe study details, title additional page(s) "Study Design" and attach them to this Worksheet.

Study designed by; _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

☐ Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section VI, B, page 3 of the Study Protocol.

WORKER SAFETY CONSIDERATIONS:

☐ Investigator should initial here to indicate that all personnel handling drug have read Material Safety Data Sheet for Chloramine-T and have been provided protective equipment, in good working condition, as described in the MSDS.

Date Prepared: _____ **Investigator:** _____

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

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

INSTRUCTIONS

1. Investigator must fill out Form CLT-1 **immediately** upon receipt of chloramine-T.
2. Investigator should keep the original on file, and send one copy to the Study Monitor for review.
3. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO.
4. **Note:** Both Investigator and Study Monitor should sign and date Form CLT-1.

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

Name of Drug	**Chloramine-T**	INAD Number	**9321**
Proposed Use of Drug	Treatment or control of bacterial gill disease or certain flavobacteriosis that occur in a variety of fish species		
Date of CVM Authorization Letter	July 11, 2003		
Date of Drug Receipt		**Amount of Drug Received**	
Drug Lot Number		**Study Worksheet Number**	
Name of Investigator			
Address of Investigator			
Location of Trial			
Pivotal Study		Non-pivotal Study (yes/no)	----
Approximate Number of Treated Animals		**Approximate Number of Control Animals**	
Number of Animals Used Previously[1]			
Study Protocol Number	9321		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	20 mg/L for 1hour		
Methods(s) of Administration	Immersion (static bath or flow-through treatment)		
Withdrawal Period	Zero		

[1] To be filled out by the NIO

Date Prepared: _____ **Investigator:** _____

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

Date Reviewed: _____ **Sponsor:** _____

Chloramine-T Clinical Field Trials
CLT-2: Drug Inventory Form - Version 4
Chloramine-T INAD 9321

INSTRUCTIONS

1. Investigator should initiate a new form CLT-2 **immediately** upon receipt of each shipment of Chloramine-T.
2. Form CLT-2 should be updated whenever drug is used, transferred, or discarded.
3. Investigator should save all copies of this form until the end of the calendar year, at which time they should maintain all originals on file and send one copy of the completed form(s) to their Study Monitor. Within 10 days of receipt, the Study Monitor will ensure accuracy and send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form CLT-2.

Qty of CLT from previous page (ml) _____ Facility _____ Reporting individual _____

Date	Amount of new CLT received (Kg)	Lot number of CLT received	Study Number	Amount CLT used in treatment (Kg)	CLT transferred (Kg)	CLT discarded (Kg)	CLT remaining on hand (Kg)	Inventory by (initials)
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						

Date Prepared: _____ **Investigator:** _____

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

Form CLT-3b: Results Report Form for use of Chloramine-T under INAD 9321 - All use excluding salmonids treated for BGD at 12 - 20 mg/L; treatment on 3 consecutive or 3 alternate days

INSTRUCTIONS

1. Investigator must fill out Form CLT-3B no later than 10 days after completion of the 14-day post-treatment observation period. Study Number must be recorded on all pages of Form CLT-3B. Attach lab reports and other information.
2. If Chloramine-T was not used under the assigned Study Number, fill out only the Site Information portion on this page, and skip to the end of page 3 and fill out only the "Negative Report" section.
3. Investigator should keep the original on file, and send a copy to the Study Monitor. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO for inclusion in the permanent file.
4. Note: Both Investigator and Study Monitor should sign and date Form CLT-3B.

SITE INFORMATION

Facility	
Reporting Individual	

TREATMENT INFORMATION AND SCHEDULE

Drug lot number		Total amount drug used (kg)			
Fish species treated		CLT dosage used (mg/L)			
Disease treated		Disease diagnosed by			
Average fish weight (gm)		Average fish length (in)			
Number of fish per unit (e.g. 10,000 fish/raceway)					
Number of treated units		Total number of treated fish			
Number of control units		Total number of control fish			
Check type of treatment	_____ Flow through _____ Standing bath				
Check treatment objective	_____ A _____ B _____ C _____ D				
Dates of treatment (disease control)	1st		2nd		3rd	
Date treatment started (disease prevention)		Date treatment ended (disease prevention)			

WATER QUALITY PARAMETERS

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

Daily Mortality Record

INSTRUCTIONS

1. Investigator should fill out the Daily Mortality Record as completely as possible.
2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is **T**reated or **C**ontrol, and the number of fish in each rearing unit.
3. Water temperature and individual tank mortality should be recorded on a daily basis.
4. If treatment is on 3 consecutive days, fill in only days 1-3 of the "treatment period" and proceed directly to day 1 of the "post-treatment period". If treatment is on 3 alternate days, fill in days 1-5 of the "treatment period" and proceed to day 1 of the "post-treatment period". If less than 3 treatments are used, proceed directly to day 1 of the "post-treatment period" after the final treatment. Please mark all treatment days with an asterisk.
5. Use additional copies of this form if more than 6 rearing units are involved in the trial.

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

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

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

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

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

DRUG DISCHARGE RESULTING FROM THIS TREATMENT: Use Addendum 2: Discharge Worksheet for calculations and attach completed Discharge Worksheet to this form. Enter the value from Addendum 2 step 3 in this space.

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

☐ Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section VI, B, page 3 of the Study Protocol.

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

NEGATIVE REPORT Chloramine-T was not used at this facility under this Study Number during the reporting period. (Investigator should initial for negative reports as soon as the Study Number is known to be no longer needed or valid.)

Date Prepared: _____ **Investigator:** _____

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