

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

Sponsor:

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

Sponsor Signature

Date Approved

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

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

Office for Coordination of Chloramine-T INAD:

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

Proposed Starting Date: September 1, 2007

Proposed Ending Date: December 31, 2026

Study Director: Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility _____

Investigator _____

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STUDY PROTOCOL FOR AN AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR 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. **Note: No clinical field trials will be conducted under this INAD for use patterns for which Chloramine-T has already received FDA-approval (e.g., treatment of BGD in freshwater-reared salmonids, treatment of external columnaris in walleye and freshwater-reared warmwater finfish (NADA 141-423)).**

II. SPONSOR:

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Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

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

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

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program
4050 Bridger Canyon Road, Bozeman, MT 59715;
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Study Monitors: See Appendix II for names and addresses.

I. INVESTIGATORS/FACILITIES:

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Reauthorization Starting Date: September 1, 2007

Reauthorization Expiration Date: December 31, 2026

V. BACKGROUND/PURPOSE:

- A. Chloramine-T is currently approved in the United States for use in for the following diseases/fish species (NADA 141-423). If your treatment is for an approved use then the INAD will not be used.
1. Freshwater-reared salmonids for control of mortality due to bacterial gill disease associated with *Flavobacterium* spp.
 2. Walleye for control of mortality due to external columnaris disease associated with *F. columnare*.
 3. Freshwater-reared warmwater finfish for control of mortality due to external columnaris disease associated with *F. columnare*.
- B. 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, aeromonads, and pseudomonads (Snieszko 1981; Post 1987). Moreover, gill lesions infected with these organisms provide excellent habitat for opportunistic fungi (Warren 1991).
- C. Flavobacteriosis - Flavobacteriosis, caused by *Flavobacterium columnare*, *Flavobacterium phycophilum*, 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 flavobacters 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, flavobacters 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).

D. 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. If an external flavobacteriosis is treated then the specific bacteria will need to be diagnosed. **Note: no clinical field trials will be conducted under this INAD for use patterns for which Chloramine-T has already received FDA-approval (e.g., treatment of BGD in freshwater-reared salmonids, treatment of external columnaris in walleye and freshwater-reared warmwater finfish (NADA 141-423)).**
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.

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

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

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

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

A copy of the label to be attached to each container of Chloramine-T are provided in Appendix V. Although investigational labels will be affixed to Chloramine-T containers by the supplier, 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. All facilities using Chloramine-T:

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

All Investigators are also responsible for maintaining an accurate inventory of Chloramine-T on-hand. A Chemical Use Log (Form CLT-2) must be completed and maintained by each Investigator. Each time Chloramine-T is used, it must be recorded by the Investigator in the Results Report form in the "Amount Of Drug Used" table.

At the conclusion of field trials, all remaining Chloramine-T will be destroyed by following the MSDS (note: unless treatment is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all Chloramine-T must be properly recorded and accounted for on the Chemical Use Log (Form CLT-2). The Study Monitor will be responsible for verifying the quantity of Chloramine-T remaining on hand versus the amount indicated on Form CLT-2. **Note:** Chloramine-T can be transferred to other facilities that are participating under INAD 9321. Transfers must be shown in the Drug Inventory section of the database (formerly 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 the Chloramine-T INAD will need to complete several forms located in the online INAD database. These forms are described in Section XIII. Copies of these forms are attached to this Study Protocol and will be used as a guide only for collecting the data that will be entered into the online INAD database.

VIII. EXPERIMENTAL UNIT

The experimental unit in these clinical field trials will consist of contained or isolated groups of fish. This 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 for the current calendar year 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). However, poor planning and/or a lack of preparation will not be considered an emergency situation.

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

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on a Form CLT-3. Drug discharge must be in compliance with local **NPDES** permitting requirements. The water quality benchmark for Chloramine-T is **0.13 ppm** or if > 0.13 ppm be in compliance with discharge levels set by the NPDES permitting agencies. Facilities are required to notify their NPDES Office of the benchmark set by the FDA environmental team. Note: sodium thiosulfate can be used to neutralize the Chloramine-T.

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

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

Prior to initiating each treatment event, the Investigator must first complete Form CLT-W. "Worksheet for Designing Individual Field Trials" (located under the "New Study Request" tab in the investigator account) that pertains to each specific treatment event. The worksheet should be filled out and forwarded to the Study Monitor through the online INAD database. The Study Monitor will review the planned treatment (worksheet) and forward it to the Study Director at the AADAP Office. The Study Director will then review the worksheet, assign the approved treatment a Study Number, and then the online INAD database will notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on any paper forms that are being used as a guide to collect the data to enter in the online database (i.e., Form CLT-2 and CLT-3), as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach the Study Monitor with regards to Worksheet approval and the need for treatment is immediate, the Investigator should contact the AADAP Office for permission to proceed.

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

A. Pathogen/disease considerations

1. 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-3 "Results Report Forms"**).
2. 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).

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

X. TREATMENT GROUPS

- A. A treatment group or experimental unit may be an entire tank, pond, raceway, group of fish, or it may be individual animals.
- B. Separately confined, untreated control fish will not be required in supplementary field studies conducted to determine the effectiveness and safety of 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.
- C. Although as stated above untreated control groups are not a required element of treatment under this INAD exemption, **it is important for all investigators to note that field trials conducted under a more stringent study protocol (i.e including requirements for non-treated controls groups, replication, blinding, dose verification, etc.) will ultimately be required in order to support a NADA for Chloramine-T. It is also important to note that the INAD sponsor fully expects that a limited number of facilities/investigators listed under this INAD exemption will agree to participate in such “pivotal” efficacy studies.** These studies will be initiated only after direct consultation between facilities/investigators and the sponsor. These studies will be conducted under a separate FDA-approved study protocol (i.e. not the INAD study protocol), and will also be conducted with assistance from, and under the direct supervision of, the sponsor. **If for any reason it becomes apparent to the sponsor that facilities/investigators listed under this INAD are not willing to participate in such “pivotal” studies, the sponsor will request that FDA terminate the INAD.**

XI. TREATMENT SCHEDULES

- A. Route of administration

Chloramine-T will 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 flavobacteriosis in salmonids, sturgeon, perch, sunfish, bass and other coolwater and warmwater fish species. The specific bacteria needs to be reported in Form CLT-3 if external flavobacteriosis is detected.]

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

Treatment duration is 1 hour (standing bath or flow-through).

Administered one day per week.

Objective B [For the treatment of BGD in salmonid fish species] **Note:** this is an approved use so an INAD will not be used for this objective.

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

Treatment duration is 1 hour (standing bath or flow-through).

Administered 3 consecutive or alternate days

Objective C [For the treatment of external flavobacteriosis in salmonid fish species. The specific bacteria needs to be reported in Form CLT-3 if external flavobacteriosis is detected.]

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

Treatment duration is 1 hour (standing bath or flow-through).

Administered 3 consecutive or alternate days

Objective D [For the treatment of BGD and external flavobacteriosis in sturgeon, perch, sunfish, bass, and other coolwater and warmwater fish species. The specific bacteria needs to be reported in Form CLT-3 if external flavobacteriosis is detected.] **Note:** there is an approved label for chloramine-T treatment of walleye and freshwater-reared warmwater finfish for treatment of *F. columnare*.

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

Treatment duration is 1 hour (standing bath or flow-through).

Administered 3 consecutive or alternate days

C. Dosing interval and repetition

Chloramine-T will be administered as a single treatment regimen, 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 minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with Chloramine-T.

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

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or other pertinent species information indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form CLT-3. 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. **Source data must be collected for 5 days before treatment, during treatment, and for 10 days after the treatment period has ended.** Collection of this data is critically important. 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.

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 has been used extensively for many years with beneficial effect in fish culture, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions. **If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.**

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

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol 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 - located in the New Study Request tab

Form CLT-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals – located in the Manage/View Drug Inventory tab

Form CLT-2. Chemical Use Log for Clinical Field Trials under Chloramine-T INAD 9321 – located in the Manage/View Drug Inventory tab and filled out in Form CLT-3 to show use

Form CLT-3. Results Report Form for Use of Chloramine-T INAD 9321 – located in the Active Studies table on the home page

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

XIV. RECORD KEEPING PROCEDURES

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

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. 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-3. Also, note that the Investigator is also requested to estimate the predicted number of days/months before treated fish will be susceptible to harvest and/or human consumption on Form CLT-3.

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. Drug disposal information is available in the Material Safety Data Sheet (MSDS) located in Appendix IV of this protocol. Disposition of all Chloramine-T must be properly recorded and accounted for on the Chemical Use Log (Form CLT-2). The Study Monitor will be responsible for verifying the quantity of Chloramine-T remaining on hand versus the amount indicated on Form CLT-2. The investigational drug may not be redistributed to others not specified by the protocol and should not be retained by the Investigator after completion of the study (note: unless Chloramine-T is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). The investigational drug may not be redistributed to others not specified in the Study Protocol. Transfers must be shown on Form CLT-2.

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

A. Drug distribution

See Section VII.A.6. Accountability 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 will be selected by each facility that is authorized to treat fish with Chloramine-T under this INAD. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the 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.).

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 for details and the following forms will be used as guides for data collection: Form CLT-W, Form CLT-1, Form CLT-2, and Form CLT-3.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitor who will ensure that all required information is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare summary reports that will be submitted to the FDA. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

G. Data storage

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

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. INAD reports will be prepared and submitted to the FDA as required. This submission may

include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before a field trial begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarded to 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.** Deviations should be documented on Form CLT-3 in the *Description of Results* section and in the *Study Deviation* field.

XXI: E.O. 13891

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

LITERATURE CITED

- 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 I. Sponsor Contact Information for Chloramine-T INAD #9321

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

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

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

**Principal Clinical Field
Trial Coordinator:**

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

1

Appendix II. Study Monitors for Chloramine-T INAD #9321

Note: This information will be provided directly to CVM

1

1Appendix IIIa. Facilities and Names of Investigators Participating under Chloramine-T INAD #9321

Note: This information will be provided directly to CVM; Syndel; and B.L. Mitchell

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

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

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

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

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

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

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

1

Appendix IV. Material Safety Data Sheet (MSDS) for Halamid® Aqua INAD #9321

The MSDS for Halamid® Aqua can be found at the drug supplier's website

[Halamid-Aqua-SDS-04-2014.pdf \(syndel.com\)](#)

1

Appendix V. Investigational Label for Chloramine-T INAD #9321

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

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

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

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

1

Appendix VIa. Fish Species Treated under Chloramine-T INAD #9321

Freshwater finfish

1

Appendix VIb. Table of Facilities and Fish Stocks Treated under Chloramine-T INAD #9321

Note: This information will be provided directly to CVM

All data must be entered through the online INAD database:

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

Chloramine-T Clinical Field Trials

CLT-W: Worksheet for Designing Study Numbers - Version 4

Chloramine-T INAD 9321

INSTRUCTIONS

- Investigator must fill out Form CLT-W for each trial conducted under this INAD **before** actual use of Chloramine-T.
- Investigator should forward a copy of CLT-W to the Study Monitor for review.
- After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of the Study Number.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Fish species to be treated		Disease to be treated (Note: If an external flavobacteriosis is treated then the specific bacteria will need to be diagnosed.)	
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		<input type="checkbox"/> Flow through <input type="checkbox"/> Standing bath	
Check type of treatment		<input type="checkbox"/> Disease control <input type="checkbox"/> Disease prevention	
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: Provide a brief description of your planned study. The description should include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

☐ 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 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

- Investigator must fill out Form CLT-1 **immediately** upon receipt of Reward®.
- Investigator should forward a copy of Form CLT-1 to the Study Director at the AADAP Office.

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

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 12, 2008		
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 (yes/no)		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: _____

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

1. Investigator should initiate a new form CLT-2 **immediately** upon receipt of each shipment of Chloramine-T.
2. Each lot number of Chloramine-T may be used for multiple treatment regimens.

Reporting
Individual[illegible]

Investigator: _____

Study Monitor:

Form CLT-3: Results Report Form for use of Chloramine-T under INAD 9321

INSTRUCTIONS

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

SITE INFORMATION

Facility	
Reporting Individual	

TREATMENT INFORMATION AND SCHEDULE

Drug lot number		Total amount drug used (kg)	
Fish species treated		CLT dosage used (mg/L)	
Disease treated (Note: If an external flavobacteriosis is treated then the specific bacteria will need to be diagnosed.)		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 (°F)		Dissolved Oxygen (mg/L)	
Ave treatment temp (°F)		pH	
Ave post-treatment temp (°F)		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 Treated or Control, and the number of fish in each rearing unit.
3. Water temperature and individual tank mortality should be recorded on a daily basis.
4. 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 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. **Even if mortality is zero an entry is still needed for that day.**

FACILITY										
	Rearing Unit ID									
	Treated or Control									
	Number of Fish									
	Day	Date	Water Temp (F°)	Mortality	Mortality	Mortality	Mortality	Mortality	Mortality	Daily Observer Initials
Pre-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: Calculate your facility's treated water effluent discharge level at the point the water reaches public waters. If water was neutralized with sodium thiosulfate indicate that here. The FDA benchmark for the discharge level is **0.13 ppm** unless your NPDES permit allows a higher discharge.

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, 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. The study will be closed out in the online INAD database.

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

NOTICES**Paperwork Reduction Act**

In accordance with the Paperwork Reduction Act (44 U.S.C. 3501 *et seq.*), the U.S. Fish and Wildlife Service collects information necessary to permit the use of an investigational new animal drug to generate data to support a new animal drug approval (NADA) as part of the Fish and Aquatic Conservation fish health network. Your response is voluntary, but is required to obtain or retain a benefit. According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. OMB has approved this collection of information and assigned Control No. 1018-####.

ESTIMATED BURDEN STATEMENT

We estimate public reporting for this collection of information to average 4 hours, including time for reviewing instructions, gathering and maintaining data, and completing and reviewing the form. Direct comments regarding the burden estimate or any other aspect of the form to the Service Information Clearance Officer, Fish and Wildlife Service, U.S. Department of the Interior, 5275 Leesburg Pike, MS: PRB (JAO/3W), Falls Church, VA 22041-3803, or via email at Info_Coll@fws.gov. Please do not send your completed form to this address.

FREEDOM OF INFORMATION ACT STATEMENT

Information provided to the Service is generally subject to release to the public under the Freedom of Information Act (FOIA). Certain information, however, may be subject to withholding if the Service determines that the information is a trade secret and/or commercial or financial information that is privileged or confidential. To the extent you are submitting business information that falls into one of these categories, you must clearly mark this information as "Business Confidential" in order for the Service to assess the applicability of FOIA Exemption 4. Any information provided by you that is not marked as "Business Confidential" will be considered releasable to the public under the FOIA [43 CFR 2.26 – 2.33].