# Chloramine-T Clinical Field Trials - INAD 4000

# Year 2000 Annual Summary Report on the Use of Chloramine-T in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U. S. Fish and Wildlife Service
Bozeman National INAD Office
Bozeman, Montana

## Summary

Chloramine-T was used at seven U. S. Fish and Wildlife Service hatcheries during the year 2000 to evaluate its efficacy to control mortality caused by bacterial gill disease, external columnaris, flavobacteriosis, and Ichthyophthirios moltitilis in sturgeon chub and several salmonid species. The U.S. Food and Drug Administration has authorized the use of chloramine-T by the Fish and Wildlife Service under Compassionate Investigational New Animal Drug Exemption #4000 for the purpose of collecting pivotal and ancillary efficacy data to support a new animal drug approval for chloramine-T. Chloramine-T was administered in 216 disease control/prevention trials and involved approximately 6.4 million fish. The compassionate study protocol under which treatments were administered allowed investigators to treat fish with chloramine-T on alternate days up to three times for 1hr at dosages ranging from 10-15 mg/L; however, in a few select studies fish were treated twice at 6.0 mg/L or treated prophylactically at 35 - 40 mg/L. Approximately 59% of trials appeared efficacious, 19% appeared ineffective, and 22% were characterized as inconclusive.

## Introduction

Bacterial gill disease (BGD) is one of the most common diseases of hatchery reared salmonids (Bullock 1990) and causes more fish losses than any other bacterial disease (Bills et al. 1988). In Ontario, Canada this disease accounts for about 21% of all diagnostic submissions from fish farms to the Fish Pathology Laboratory of the Ontario Veterinary College (Ferguson et al. 1991). Fish mortality is generally not a direct result of the infection, but is a consequence of the infection. Mortality is most likely the result of asphyxiation from lack of adequate oxygen exchange in severely congested gills. Stressors associated with intense fish culture may predispose fish to infection. Although *Flavobacterium branchiophilum* is the bacteria responsible for causing most outbreaks of BGD (Wakabayashi, H, et al., 1989; Ferguson et al., 1991), other gram-

negative bacteria have also been implicated. Proliferation of gill epithelial tissue, and later the loss of gill surface by clubbing and fusing of lamellae are often associated with this bacterial infection (Bullock 1990). The disease is characterized by acute onset, flared opercula, increased branchial rate, decreased fright response, equidistant spacing of infected animals, reduced food consumption, and high mortality (Lumsden et al. 1994; Lasee 1995; Post 1987). Clinical signs of BGD have been well documented, and it is widely known that this disease can cause the rapid proliferation of gill epithelium and the production of excess mucus as the host responds defensively to the infection. This response can "smother" gills and cause severe fish losses if prompt measures are not taken. If BGD, which is horizontally transmitted, is not diagnosed and treated early, an epizootic may occur within a 24-h period (Bullock et al. 1990).

As previously mentioned, *F. branchiophilum* is the bacteria responsible for most outbreaks of BGD. However, other gram-negative bacteria have also been implicated. These “other” bacteria include *F. aquatile, F. psychrophilus, F. columnaris,* as well as other flavobacters including some aeromonads and pseudomonads. External bacterial infections related to bacterial cold water disease (CWD), caused by *F. psychrophilus*, are grouped in this category of "other" bacteria, which when external, may cause BGD-like symptoms. Bacterial coldwater disease, like BGD, is caused by long, thin, filamentous bacteria that produce yellow pigment on artificial media. Without careful bacteriological or serological work it is often difficult to accurately determine the identity of this bacteria. In some cases, BGD may be complicated by the occurrence of systemic infections caused by other bacteria including F. *psychrophilus* and *Aeromonas salmonicida*.

Historically, several chemicals including benzalkonium chloride (available as Hyamine 1622 and 3500), diquat, and chloramine-T have been used to control mortality caused by BGD (Bullock et al. 1990). However, none of these chemicals have been approved by the FDA to control mortality in freshwater fish caused by BGD. Because chloramine-T appears to be the most effective therapeutant when salmonids have BGD (From 1980; Bullock et al. 1990) it has become the prime candidate for approval with the FDA as a bath treatment. Chloramine-T has been characterized as a non-selective sanitizing agent and has been shown to clean up gills infested with bacteria and coated with excess mucus. Ancillary efficacy data compiled by the U.S. Fish & Wildlife Service in previous years under INAD 4000 have indicated that chloramine-T administered at 10 or 15 mg/L for 1 hr using a flow through or standing bath treatment on three alternate days is an effective treatment regime for BGD (Bowker and Erdahl 1998).

## Purpose of Report

The primary purpose of this report is to summarize the results of calendar year (CY) 2000 supplemental chloramine-T field efficacy data. However, it is also expected that these data will be used to enhance the existing chloramine-T database that has been established from previous years for the purpose of developing an appropriate label

claim for the use of this new animal drug.

## Facilities, Materials, Treatment Procedures

### 1. Facilities

A total of 7 U.S. Fish and Wildlife Service National Fish Hatcheries (NFH) used chloramine-T to control/prevent mortality caused by BGD, external columnaris, flavobacteriosis, or Ichthyophthirios moltitilin.

### 2. Chemical material

Chloramine-T (CAS No. 127-65-1) is a pure white crystal powder. All facilities used designated lots of chloramine-T provided by the manufacture, Akzo Chemical, Inc., Denver, CO.

### 3. Treatment Methods

Chloramine-T treatment was administered using either flow-through or standing bath treatments. Both procedures called for accurately weighed amounts of dry chemical to be dissolved in an appropriate amount of non-chlorinated water. When using a flow-through system, dissolved chemical was metered into rearing units at a rate to achieve the desired treatment concentration during a 1 hr period. When using a standing bath method, water flow to the rearing unit was turned off and dissolved chemical added to the rearing unit and mixed thoroughly to ensure uniform chloramine-T concentration throughout the tank. Thorough mixing was essential to ensure there were no chloramine-T "hot spots." After the 1 hr treatment, water flow was turned on again to flush the chemical out of the rearing unit.

### 4. Drug dosages

Chloramine-T was used at concentrations ranging from 6 - 40 mg/L. During CY 2000, a dosage of 6.0 mg/L was administered in 2 trials; 10 mg/L in 129 trials; 10 mg/L on first day of treatment and then 15 mg/L for second and third day of treatment in 6 trials; 12 mg/L in 1 trial; 15 mg/L in 77 trials; and 35 - 40 mg/L in 1 trial.

### 5. Number of treatments per disease outbreak

According to the Study Protocol, Investigators were allowed to administer chloramine-T up to 3 times on alternate days when used to control mortality caused by BGD, and up to three times per week when used to prevent mortality. Chloramine-T was used 1, 2 or 3 times to control mortality, and was typically

used intermittently to prevent mortality. Intermittent use followed no predetermined treatment schedule, and occurred in 8 trials at the Alchesay/Williams Creek and Ennis NFHs. In these trials, fish were treated at the first indication of behavior characteristic of flavobacteriosis or BGD (as documented by Post, 1987; Lumsden et al., 1994; and Lasee, 1995).

## Fish Species Treated and Fish Diseases Involved in 2000 Trials

### 1. Species and size of fish treated

Four different fish species were treated during CY 2000. Species treated included rainbow trout (*Oncorhynchus mykiss*); cutthroat trout (*O. clarki*); apache trout *(O. apache)*; and sturgeon chub (*Macrhybopsis gelida*). Approximately 89% of salmonids treated were less than 5" in length.

### 2. Diseases treated

The disease treated most frequently was characterized as BGD. Other diseases treated included columnaris, external flavobacteriosis, and Ichthyophthirios moltitilis.

## Data Collected

### 1. Pathologist's report

Although no pathologist's reports were submitted in CY 2000, pathologist's reports would certainly have enhanced the quality of data submitted. Such information typically includes: 1) a description of how the identity of disease agent(s) was verified; 2) copies of a pathology report or the disease identification records that confirm the presence of the disease agent; and 3) the name and title of the individual performing the diagnosis. Additionally, evidence would typically be provided to document that there were no secondary infections or infestations caused by unrelated disease agents in the population of test fish.

### 2. Treatment response and drug accountability data

Drug receipt reports, drug use reports, diagnosis, treatment, and mortality reports (including adverse effects/toxicity observations), and fish disposition reports were prepared by the Investigators. These reports were routed through the Study Monitor for review, and then sent to the Bozeman National INAD Office for review, data analysis, data basing, and storage in permanent files.

The collection of accurate daily mortality data is essential for evaluation of

efficacy and adverse effects/toxicity. The Study Protocol states that data should be collected 10 d prior to treatment, during treatment, and for 14 d post-treatment. However, for a variety of reasons, mortality data was not always collected for this entire period. Reasons for incomplete mortality data include: 1) splitting of fish into additional rearing units; and 2) stocking of early life stage fish shortly after final treatment. Stocking of early life stage fish before the withdrawal period had elapsed was allowed as fish would not be harvestable for several months, thereby complying with the established withdrawal period.

## Discussion of Study Results

### 1. Relevance of study to a proposed label claim for chloramine-T

Results of CY 2000 trials for the most part supported the findings detailed in the 1995 - 1999 Annual Reports on the Use of Chloramine-T Under INAD #4000. A proposed label claim for chloramine-T is described in Appendix I.

### 2. Observations on the efficacy of chloramine-T

#### A. Efficacy at 6 mg/L chloramine-T

A total of 2 outbreaks of presumptively diagnosed BGD were treated with 6 mg/L chloramine-T (Tables 1). Both of these trials appeared to be efficacious. The fish species treated was apache trout.

#### B. Efficacy at 10 mg/L chloramine-T

A total of 129 outbreaks of presumptively diagnosed BGD were treated with 10 mg/L chloramine-T (Tables 1-3). A total of 68 of these trials appeared efficacious, while 32 trials did not appear efficacious, and 29 trials were characterized as inconclusive. Fish species treated included rainbow trout and cutthroat trout.

#### C. Efficacy at 10 mg/L chloramine-T on first day of treatment, and 15 mg/L chloramine-T on second and third day

A total of 6 outbreaks of presumptively diagnosed columnaris were treated with 10 mg/L chloramine-T on the first day of treatment, and 15 mg/L chloramine-T on second and third day (Tables 2 - 3). A total of 2 of these trials did not appear to be efficacious, while 4 trials were characterized as inconclusive. Fish species treated was cutthroat trout.

**D. Efficacy at 12 mg/L chloramine-T**

A single outbreak of presumptively diagnosed BGD was treated with 12 mg/L chloramine-T (Table 3). This trial was characterized as inconclusive as fish may have had a secondary internal infection as well as BGD. Fish species treated was the rainbow trout.

**E. Efficacy at 15 mg/L chloramine-T**

A total of 77 outbreaks of presumptively diagnosed BGD and flavobacteriosis were treated with 15 mg/L chloramine-T (Tables 1-3). A total of 56 of these trials appeared efficacious, 8 trials did not appear efficacious, and 13 trials were characterized as inconclusive. Fish species treated included rainbow trout, cutthroat trout, and apache trout.

**D. Efficacy at 35 - 40 mg/L chloramine-T**

A single outbreak of presumptively diagnosed Ichthyophthirios moltitilis was treated with 35 - 40 mg/L chloramine-T (Table 1). This trial appeared to be efficacious. The only fish species treated at this treatment level was the sturgeon chub. The Investigator noted that fish were treated twice a day for a duration of 2 hrs each time. It was also noted that the reason for the significant deviation from the Study Protocol was due to a research exemption (i.e. all fish were destroyed upon completion of the trial).

**3. Observed Toxicity**

No toxicity or adverse effects relating to chloramine-T treatments were reported.

## Summary of Study Results

Chloramine-T was used at dosages ranging from 6 - 40 mg/L in 216 trials. Fish were treated one, two, or three times on alternate days for 1-2 hrs. Four different species of fish were treated, and trials involved approximately 6.4 million fish. Treated fish ranged in size from 0.75 - 8.61in. Water temperature during treatment ranged from 44.5 - 56.0$^{o}$F, with a mean treatment temperature of 54.1$^{o}$F. Approximately 59% of trials appeared efficacious, 19% appeared ineffective, and 22% were characterized as inconclusive. Data from the CY 2000 trials support the results of previous Annual Report submissions under INAD #4000 that indicate that chloramine-T treatment is efficacious for the treatment of BGD and external flavobacteriosis in a variety of fish species. Also as reported in previous submissions, treatment efficacy appeared to be highest when chloramine-T dosage was 10-15 mg/L. Furthermore, investigators reported no evidence of toxicity or adverse effects related to chloramine-T treatment. However, as has been the case with previous Annual Report submissions under INAD #4000, it should be

noted no trials involved untreated control fish. Consequently, it is understood that these data must be considered as ancillary data, and that pivotal efficacy studies are needed to definitively demonstrate chloramine-T efficacy for the treatment of BGD. However, the ancillary data described above should provide useful, corroborative data to help support a label claim for the use of chloramine-T to control mortality associated with BGD in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #4000 will also be ancillary data, efforts will be made to improve the quality of data whenever possible, with particular attention paid to the use of untreated control fish, dose verification, and the inclusion of fish pathology reports.

## References


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## Table 1. Summary of Year 2000 Chloramine-T Efficacy Results - Efficacious Studies

| Hatchery | Number of efficacious trials | Fish Size (in.) | Fish Species | Number of Fish | Disease | Number of treatment days | Dose (mg/L) | Temp. (°F) |
|---|---|---|---|---|---|---|---|---|
| Alchesay-Williams Creek NFH | 2 | 7.8 - 8.2 | APT | 32,697 | BGD | 2 | 6 | 51.0 |
| Hotchkiss NFH | 2 | 1.1 - 1.7 | RBT | 63,242 | BGD | 1 | 10 | 56.0 |
| Hotchkiss NFH | 1 | 6.8 | RBT | 26,747 | BGD | 1 | 10 | 56.0 |
| Hotchkiss NFH | 20 | 1.5 - 3.6 | RBT | 694,933 | BGD | 2 | 10 | 56.0 |
| Hotchkiss NFH | 7 | 4.0 - 5.7 | RBT | 222,200 | BGD | 2 | 10 | 56.0 |
| Hotchkiss NFH | 14 | 1.5 - 3.6 | RBT | 536,034 | BGD | 3 | 10 | 56.0 |
| Hotchkiss NFH | 24 | 4.0 - 5.4 | RBT | 669,794 | BGD | 3 | 10 | 56.0 |
| Alchesay-Williams Creek NFH | 1 | 2.64 | APT | 9,814 | BGD | 1 | 15 | 44.5 |
| | 18 | 2.0 - 3.0 | APT | 295,078 | BGD | 1 | 15 | 51.0 |
| | 2 | 6.80 | APT | 18,600 | BGD | 1 | 15 | 51.0 |
| | 2 | 4.2 - 4.3 | RBT | 134,976 | BGD | 1 | 15 | 51.0 |
| | 11 | 1.5 - 2.9 | APT | 124,996 | BGD | 2 | 15 | 51.0 |
| | 1 | 3.48 | CUT | 97,500 | BGD | 2 | 15 | 51.0 |
| | 2 | 3.77 | RBT | 93,822 | BGD | 2 | 15 | 51.0 |

## Table 1. Summary of Year 2000 Chloramine-T Efficacy Results - Efficacious Studies - continued

| Hatchery | Number of efficacious trials | Fish Size (in.) | Fish Species | Number of Fish | Disease | Number of treatment days | Dose (mg/L) | Temp. (°F) |
|---|---|---|---|---|---|---|---|---|
| Alchesay-Williams Creek NFH | 7 | 1.4 - 2.3 | APT | 86,693 | BGD | 3 | 15 | 51.0 |
| | 2 | 2.25 | RBT | 34,720 | BGD | 3 | 15 | 51.0 |
| Norfork NFH | 1 | 1.25 | RBT | 88,000 | BGD | 3 | 15 | 52.0 |
| | 1 | 3.01 | RBT | 113,000 | BGD | 3 | 15 | 51.1 |
| | 4 | 5.0 - 6.7 | RBT | 151,000 | BGD | 3 | 15 | 51 - 52 |
| | 1 | 8.37 | RBT | 113,000 | BGD | 3 | 15 | 51.1 |
| Ennis NFH | 3 | 5.6 - 5.8 | RBT | 3,065 | Flavobacteriosis | 7 | 15 | 54.0 |
| Bozeman FTC | 1 | 2.00 | STC | 19 | ICH | 28 | 35 - 40 | 54.0 |

## Table 2. Summary of Year 2000 Chloramine-T Efficacy Results - Non-efficacious Studies

| Hatchery | Number of non-efficacious trials | Fish Size (in.) | Fish Species | Number of Fish | Disease | Number of treatment days | Dose (mg/L) | Temp. (ºF) |
|---|---|---|---|---|---|---|---|---|
| Garrison Dam NFH | 2 | 4.50 | CUT | 20,000 | Columnaris | 3 | 10 - 15 | 53.0 |
| Hotchkiss NFH | 18 | 1.3 - 3.6 | RBT | 422,585 | BGD | 1 | 10 | 56.0 |
| | 10 | 1.5 - 2.3 | RBT | 233,419 | BGD | 2 | 10 | 56.0 |
| | 2 | 4.5 - 4.6 | RBT | 74,400 | BGD | 3 | 10 | 56.0 |
| | 2 | 2.20 | RBT | 162,000 | BGD | 10 | 10 | 56.0 |
| Alchesay-Williams Creek NFH | 2 | 1.3 - 1.8 | APT | 59,657 | BGD | 1 | 15 | 56.0 |
| | 1 | 1.63 | RBT | 31,287 | BGD | 1 | 15 | 56.0 |
| | 5 | 1.6 - 2.0 | APT | 112,865 | BGD | 3 | 15 | 56.0 |

## Table 3. Summary of Year 2000 Chloramine-T Efficacy Results -Inconclusive Studies

| Hatchery | Number of inconclusive trials | Fish Size (in.) | Fish Species | Number of Fish | Disease | Number of treatment days | Dose (mg/L) | Temp. (°F) |
|---|---|---|---|---|---|---|---|---|
| Garrison Dam NFH | 4 | 4.50 | CUT | 40,000 | Columnaris | 3 | 10 - 15 | 53.0 |
| Hotchkiss NFH | 14 | 1.1 - 2.6 | RBT | 518,515 | BGD | 1 | 10 | 56.0 |
| | 13 | 1.5 - 2.5 | RBT | 471,522 | BGD | 2 | 10 | 56.0 |
| | 1 | 3.20 | RBT | 42,000 | BGD | 3 | 10 | 56.0 |
| Garrison Dam NFH | 1 | 0.75 | CUT | 60,000 | BGD | 3 | 10 | 52.0 |
| Creston NFH | 1 | 2.71 | RBT | 85,000 | BGD | 3 | 12 | 51.0 |
| Alchesay-Williams Creek NFH | 2 | 2.4 - 2.6 | RBT | 68,089 | BGD | 1 | 15 | 51.0 |
| | 1 | 1.70 | APT | 12,215 | BGD | 2 | 15 | 51.0 |
| | 4 | 1.1 - 2.0 | APT | 88,590 | BGD | 3 | 15 | 51.0 |
| Norfork NFH | 5 | 4.5 - 5.9 | RBT | 194,500 | BGD | 3 | 15 | 51 - 52 |
| | 1 | 8.61 | RBT | 45,000 | BGD | 3 | 15 | 52.0 |

## Table 4. Summary Data Regarding Year 2000 Chloramine-T Efficacy Studies

**Total Number of Fish Treated:** **6,351,574**

| | |
|---|---|
| Number of fish treated in efficacious studies | 3,609,930 |
| Number of fish treated in non-efficacious studies | 1,116,213 |
| Number of fish treated in inconclusive studies | 1,625,431 |

**Total Number of Rearing Units Treated:** **216**

| | |
|---|---|
| Rearing Units in Efficacious Studies | 127 |
| Rearing Units in Non-efficacious Studies | 42 |
| Rearing Units in Inconclusive Studies | 47 |

**Treatment Regimes and Frequency Used:**

| | | | |
|---|---|---|---|
| 6 mg/L - three times | 2 trials | 12 mg/L - three times | 1 trial |
| 10 mg/L - one time | 35 trials | 15 mg/L - one time | 28 trials |
| 10 mg/L - two times | 50 trials | 15 mg/L - two times | 15 trials |
| 10 mg/L - three times | 42 trials | 15 mg/L - three times | 31 trials |
| 10 mg/L - ten times | 2 trials | 15 mg/L - seven times | 3 trials |

| | |
|---|---|
| 10 - 15 mg/L - three times | 6 trials |
| 35 - 40 mg/L - twenty-eight times | 1trial |

**Treatment Water Temperature ($^{o}$F):**

| | |
|---|---|
| Temperature Range | 44.5 - 56.0 |
| Mean Temperature | 54.1 |

**Size of Treated Fish (in.):**

| | |
|---|---|
| Size Range | 0.75 - 8.61 |

**Species Treated:** apache trout (*Oncorhynchus apache*)
cutthroat trout (*O. clarki*)
rainbow trout (*O. mykiss*)
sturgeon chub (*Macrhybopsis gelida*)

# Appendix I

**1. Relevance of study to a proposed label claim for chloramine-T–** Results from CY 2000 help to further define an appropriate and inclusive label claim, and clarify fish culture "use instructions" that may enhance the drug's efficacy. The following proposed label claim is recommended.

**Indications:** For the prevention and control of mortality in freshwater fish susceptible to BGD, flavobacteriosis, external columnaris, or other external bacterial diseases.

**Directions for Use**:

**Disease Prevention:** Treatment for young of the year fish at a concentration of 10 - 20 mg/L, in a 1hr standing bath or flow through immersion treatment, no more than once per day and no more than three times per week.

**Disease Control:** Treat fish susceptible to BGD, external columnaris, or flavobacteriosis, of any size, at a concentration of 10 - 20 mg/L, in a 1hr standing bath or flow through immersion treatment, no more than once per day on three alternate days.

**Preparations and Precautions Before Treatment of Fish:**

*Do not feed fish on the day(s) treatment(s) are administered. Cessation of feeding immediately reduces the fish's metabolic demands for oxygen and slows the production of toxic waste products. Feeding may be resumed on the day following treatment. Healthy fish will regain any growth lost by withholding feed for short periods.*

*Clean all rearing units to be treated and remove and count all dead and moribund fish. This process removes waste that demand oxygen during their breakdown, removes fish that may shed or carry infectious pathogens, and eliminates organic material that may reduce the effectiveness of chloramine-T*

*Consider using dosages of 10 - 15 mg/L chloramine-T when treating fish, as lack of efficacy has been shown in many cases when less then 10 mg/L chloramine-T is used.*

*To ensure sick fish have adequate oxygen, consider using mechanical aeration if the*

*standing bath treatment is to be used. Mechanical aeration will help to disperse the chemical and prevent potential "hot spots".*

*Double check all rearing unit dimensions, water depths, inflow rates (if using a flow through treatment) and numerical calculations used to determine quantity of chloramine-T to use.*

*Double check flow rates on all constant flow devices metering dissolved chemical into inflow for flow through treatments.*

*Dissolve chloramine-T in volume of water at least several times more than the volume needed to dissolve the chemical according to the solubility information on the Material Safety Data Sheet (MSDS) for chloramine-T. This information states that the solubility of chloramine-T at 25°C is 150 g per L of water. To ensure all chloramine-T is dissolved, use more water than what is needed to dissolve the amount to be used, paying particular attention to the fact that more water will be needed if colder than 25°C, as will be the case in nearly all trials. All individuals handling chloramine-T should be familiar with the contents of the MSDS for chloramine-T and be equipped with required safety gear.*

*If fish are overcrowded, split fish populations a few days after treatment has been completed and fish are once again actively feeding. Do not feed fish on the day that they are handled.*