Chloramine-T Clinical Field Trials - INAD 4000

1998 Annual Summary Report on the Use of Chloramine-T in Clinical Field Efficacy Trials

Prepared by:

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

Summary

Chloramine-T was used at nine U. S. Fish and Wildlife Service hatcheries during 1998 to evaluate its efficacy to control mortality caused by bacterial gill disease and external flexibacteriosis in paddlefish, lake sturgeon, and several species of salmonids. 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 193 disease control/prevention trials and involved approximately 7.4 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 8-15 mg/L. Approximately 68% of trials appeared efficacious, 11% appeared ineffective, and 21% 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 candidates for approval with the FDA as a bath treatment. Chl-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 1998 (CY98) 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 9 U.S. Fish and Wildlife Service (FWS) National Fish Hatcheries (NFH) used chloramine-T to control/prevent mortality caused by BGD or external flexibacteriosis.

2. Chemical material

Chloramine-T (CAS No. 127-65-1) is a pure white crystal powder. Hatcheries used designated lots of chloramine-T provided by the manufacture (Akzo Chemical, Inc.) and distributed by Western Chemical, Inc. (Ferndale, Washington).

3. Treatment Methods

Chloramine-T treatment was administered using either flow-through or a 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 8-15 mg/L. During CY98, a dosage of 8 mg/L was administered in 3 trials; 9 mg/L in 24 trials; 10 mg/L in 100 trials; 12 mg/L in 13 trials; and 15 mg/L in 53 trials.

5. Number of treatments per disease outbreak

According to the Study Protocol, Investigators were allowed to administer chloramine-T up to 3 times when used to control mortality caused by BGD, and up to three times per week when used to prevent mortality. Chl-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 fifteen trials at the Hotchkiss and Jones Hole NFHs. In these trials, fish were treated at the first indication of behavior characteristic of BGD (as documented by Post, 1987; Lumsden et al., 1994; and Lasee, 1995).

Fish Species Treated and Fish Diseases Involved in 1998 Trials

1. Species and size of fish treated

Eight different fish species were treated during CY98. Species treated included: rainbow trout (*Oncorhynchus mykiss*); cutthroat trout (*O. clarki*); kokanee salmon (*O. nerka*); apache trout *(O. apache)*; steelhead trout *(O. mykiss)*; brown trout *(Salmo trutta);* paddlefish *(Polydon spathula)*; and lake sturgeon *(Acipenser fluvescens)*. Approximately 91% of salmonids treated were less than 5" in length.

2. Diseases treated

The disease treated most frequently was characterized as BGD.

Data Collected

1. Pathologist's report

Although no pathologist's reports were submitted in CY 98, 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) the splitting of fish into additional rearing units; and 2) the 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 98 trials for the most part confirmed the findings detailed in the 1995, 96, & 97 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 for the control of bacterial gill disease or external flexibacteriosis--

A. Efficacy at 8 mg/L chloramine-T

A total of 3 outbreaks of presumptively diagnosed cases of BGD were treated with 8 mg/L chloramine-T. In each case, chloramine-T was administered a single time (Table 1). All trials were conducted on cutthroat trout swim-up fry at the Creston NFH. The trials were characterized as inconclusive because BGD appeared as a secondary infection while the fish were being treated for suspected bacterial coldwater disease (CWD). It was later confirmed by the Bozeman Fish Health Lab that these fish had a systemic bacterial infection.

B. Efficacy at 9 mg/L chloramine-T

A total of 24 outbreaks of presumptively diagnosed cases of BGD/ filamentous, gram-negative rod-shaped bacteria were treated with 9 mg/L chloramine-T (Table 1). All trials involved steelhead trout at the Coleman NFH. In all trials chloramine-T was administered on two alternate days. Treatment was characterized as inconclusive in all trials because raceways were either split before and after treatment, or combined after treatments. The Investigator noted that fish health evaluations on day 1 and 20 of the post-treatment period showed filamentous gram negative bacteria present on 40% and 60% of fish gills samples, respectively. Chronic mortality continued post-treatment.

C. Efficacy at 10 mg/L chloramine-T

Chloramine-T was used at a dosage of 10 mg/L in 100 of 193 trials (52%; Tables 1-3). A total of 75 of these trials appeared efficacious in controlling/preventing mortality caused by BGD and external flexibacteriosis. Efficacious results were obtained when a single raceway of lake sturgeon (mean length = 0.80 in) diagnosed with columnaris were treated with chloramine-T three times. It is important to note that these results suggest that mortality caused by columnaris can be controlled if treatment is administered before the disease becomes systemic (as was suspected in this case). Other fish species treated with efficacious results included kokanee salmon, rainbow trout, and brown trout. Seventeen trials conducted using a treatment dosage of 10 mg/L chloramine-T did not appear efficacious, and 8 trials were characterized as inconclusive.

D. Efficacy at 12 mg/L chloramine-T

Chloramine-T was used at a dosage of 12 mg/L in 13 of the 193 trials (7%; Table 3). All treatments appeared efficacious. Fish species treated included rainbow trout, apache trout, and cutthroat trout.

E. Efficacy at 15 mg/L chl-T

Chloramine-T was used at a dosage of 15 mg/L in 53 of 93 trials (27%; Tables 1-3). A total of 43 trials appeared efficacious. Efficacious trials were conducted on rainbow trout, apache trout, lake sturgeon, and paddlefish. A total of 4 trials did not appear efficacious, and 6 trials were characterized as inconclusive.

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 8-15 mg/L in 193 trials. Fish were treated one, two, or three times on alternate days for 1 hr. Eight different species of fish were treated, and trials involved approximately 7.4 million fish. Treated fish ranged is size from 0.8-10.4 in. Water temperature during treatment ranged from 8.3-19.4^{o}C, with a mean treatment temperature of 12.6^{o}C. Approximately 68% of trials appeared efficacious, 11% appeared ineffective, and 21% were characterized as inconclusive. Data from the CY 98 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 flexibacteriosis 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 pathologist reports.

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Table 1. Summary of 1998 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. (°C)
Creston NFH	3	0.90	CUT	111,660	BGD	1	8	8.3
Coleman NFH	24	1.3 - 1.8	STT	461,548	BGD	2	9	10.72
Hotchkiss NFH	4	1.4 - 1.5	RBT	345,135	BGD	1	10	13.3
Hotchkiss NFH	3	3.1 - 3.2	RBT	119,644	BGD	1	10	13.3
Hotchkiss NFH	1	2.3	RBT	35,343	BGD	3	10	13.3
Norfork NFH	1	2.9	RBT	121,000	BGD	1	15	10.1
Norfork NFH	1	5.0	RBT	27,800	BGD	2	15	11.7
Neosho NFH	4	5.0	PAH	5,000	Columnaris	3	15	19.4

Table 2. Summary of 1998 Chloramine-T Efficacy Results - Non-efficacious Studies

Hatchery	# of non-efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Temp. (°C)
Hotchkiss NFH	4	2.8 & 5.9	RBT	236,024	BGD	2	10	13.3
Alchesay/Williams Creek NFH	12	2.5 - 2.8	RBT	299,894	BGD	3	10	11.1
	1	4.2	RBT	16,394	BGD	3	10	18.3
Norfork NFH	1	6.1	RBT	19,500	BGD	1	15	11.7
Alchesay/Williams Creek NFH	3	2.2 - 2.4	APT	40,740	BGD	3	15	11.1

Table 3. Summary of 1998 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. (°C)
Hotchkiss NFH	53	1.0 - 3.3	RBT	3,004,645	BGD	1	10	13.3
Hotchkiss NFH	2	3.5 - 5.1	RBT	92,945	BGD	2	10	13.3
Hotchkiss NFH	10	3.5 - 5.1	RBT	398,996	BGD	3	10	13.3
Creston NFH	5	0.90	KOE	372,000	BGD	3	10	8.3
Garrison Dam NFH	1	0.80	BNT	30,000	BGD	3	10	10.6
	2	0.80	BNT	60,000	BGD	3	10	11.1
	1	6.1	RBT	39,000	BGD	3	10	12.2
Neosho NFH	1	0.80	LST	5,000	Columnaris	3	10	17.2
Alchesay/Williams Creek NFH	1	4.2	RBT	16,394	BGD	1	12	18.3
Creston NFH	1	2.1	CUT	13,000	BGD	1	12	8.3
Alchesay/Williams Creek NFH	11	2.0 - 2.5	APT	118,645	BGD	3	12	11.1
Jones Hole NFH	1	10.4	RBT	5,000	Fin Rot/ Suspected Peduncle Disease Flexibacter	1	15	12.2
Neosho NFH	1	0.80	LST	5,000	Columnaris	1	15	17.2

Table 3. Summary of 1998 Chloramine-T Efficacy Results - Efficacious Studies (cont.)

Hatchery	Number of efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Temp. (°C)
Norfork NFH	8	2.0 - 5.7	RBT	343,500	BGD	1	15	10.1
	9	1.4 - 3.8	RBT	150,700	BGD	1	15	10.6
	2	5.5 & 7.2	RBT	56,800	BGD	1	15	11.2
	5	2.2 - 6.0	RBT	241,500	BGD	1	15	12.2
	2	5.3 - 6.5	RBT	50,000	BGD	1	15	12.8
Erwin NFH	1	9.0	RBT	4,000	BGD	2	15	13.5
Norfork NFH	1	8.0	RBT	15,000	BGD	2	15	10.6
	3	5.2 - 5.6	RBT	94,500	BGD	2	15	11.7
Alchesay/Williams Creek NFH	2	2.0 - 2.5	APT	24,931	BGD	3	15	11.1
	2	2.0 - 4.0	RBT	29,332	BGD	3	15	11.1
Neosho NFH	4	5.0	PAH	5,000	Columnaris	3	15	19.4
Norfork NFH	2	6.0 - 6.1	RBT	53,000	BGD	3	15	11.7

Table 4. Summary Data Regarding 1998 Chloramine-T Efficacy Studies

Total Number of Fish Treated:	**7,407,870**
Number of fish treated in efficacious studies	5,577,859
Number of fish treated in non-efficacious studies	597,881
Number of fish treated in inconclusive studies	1,232,130
Total Number of Rearing Units Treated:	**193**
Rearing Units in Efficacious Studies	131
Rearing Units in Non-efficacious Studies	24
Rearing Units in Inconclusive Studies	38

Treatment Regimes and Frequency Used:

8 mg/L - one time	3 trials
9 mg/L - two times	24 trials
10 mg/L - one time	60 trials
10 mg/L - two times	6 trials
10 mg/L - three times	34 trials
12 mg/L - one time	2 trials
12 mg/L - three times	11 trials
15 mg/L - one time	30 trials
15 mg/L - two times	6 trials
15 mg/L - three	17 trials

Treatment Water Temperature (^{o}C):

Temperature Range	8.3 - 19.4
Mean Temperature	12.6

Size of Treated Fish (in.):

Size Range	0.80 - 10.4

Species Treated:
rainbow trout (*Oncorhynchus mykiss*)
cutthroat trout (*O. clarki*)
kokanee salmon (*O. nerka*)
apache trout (*O. apache*)
steelhead trout (*O. mykiss*)
brown trout (*Salmo trutta*)
paddlefish (*Polydon spathula*)
lake sturgeon (*Acipenser fluvescens*)

Appendix I

1. Relevance of study to a proposed label claim for chloramine-T– Results from CY 98 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, external flexibacteriosis, or other external bacterial diseases.

Directions for Use:

Disease Prevention: Treatment for young of the year fish at a concentration of 8 - 15 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 or external flexibacteriosis, of any size, at a concentration of 8 - 15 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.