Chloramine-T Clinical Field Trials - INAD 9321

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

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Summary

Chloramine-T was used at 9 state fish hatcheries, 5 private hatcheries, and 3 tribal hatcheries during year 2000 to evaluate its efficacy to control mortality caused by bacterial gill disease, external flavobacteriosis, external columnaris, and external bacterial coldwater disease in several species of salmonids and non-salmonids. The U.S. Food and Drug Administration has authorized the use of chloramine-T by the U.S. Fish and Wildlife Service under Compassionate Investigational New Animal Drug Exemption #9321 for the purpose of collecting ancillary efficacy data to support a new animal drug approval for chloramine-T. Chloramine-T was administered in 78 disease control/prevention trials and involved approximately 19.2 million fish. The compassionate study protocol under which treatments were administered allowed the investigator to use chloramine-T on consecutive days up to three times for 1hr at dosages ranging from 10 - 30 mg/L. Approximately 68% of the trials appeared efficacious, while 6% appeared ineffective, and 26% 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 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 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 cold water 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 U.S. Food and Drug Administration (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 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 9 state fish hatcheries, 5 private hatcheries, and 3 tribal hatcheries used chloramine-T to control/prevent mortality caused by BGD, external flavobacteriosis, external columnaris, and external bacterial coldwater disease.

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. During CY 2000, a total of 939.558 kg of chl-T was used in 78 trials.

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 10 - 30 mg/L. During CY 2000, a dosage of 10 mg/L was administered in 6 trials, 14.68 mg/L in 2 trials, 15 mg/L in 28 trials, 20 mg/L in 39 trials, 10 & 15 mg/L in 1 trial, 10, 15, & 20 mg/L in 1 trial, and 20 & 30 mg/L in 1 trial.

5. Number of treatments per disease outbreak

According to the Study Protocol, Investigators were allowed to administer chloramine-T on up to 3 consecutive days when used to control mortality caused by BGD, and once a week when used to prevent mortality. Chl-T was typically used for 3 consecutive days to control mortality.

Fish Species Treated and Fish Diseases Involved in CY 2000 Trials

1. Species and size of fish treated

Twelve different fish species were treated during CY 2000. Species treated included brook trout (*Salvelinus fontinalis*),rainbow and steelhead trout (*Oncorhynchus mykiss*), chinook salmon *(O. tshawytscha)*, chum salmon (*O. keta*), sockeye and kokanee salmon (*O. nerka),* muskie (*Esox masquinongy*),

tiger muskie (*muskellunge x northern pike)*, bluegill (*Lepomis macrochirus*), goldfish (*Carassius auratus*), and white seabass (*Atractoscion nobilis*).

2. Diseases treated

The disease treated most frequently was characterized as BGD. Other diseases treated were: external flavobacteriosis, external columnaris, and external bacterial coldwater disease

Data Collected

A summary of all chloramine-T studies conducted during CY 2000 under INAD #9321 is presented in Table 5.

1. Pathologist's report

In CY 2000 approximately 68% of the reports submitted contained a pathologist's report, as compared to CY 99, when only about 28% of the reports included a pathology report. Additional 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 5 d prior to treatment, during treatment, and for 20 d post-treatment. However, for a variety of reasons, mortality data was not always collected for this entire period. Reasons for incomplete mortality data included : 1) splitting fish into additional rearing units; and 2) stocking 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 conducted under INAD #9321 largely confirmed the findings detailed in previous reports submitted to FDA under INAD #9321, as well as, 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 10 mg/L chloramine-T

A total of 6 outbreaks of presumptively diagnosed cases of BGD were treated with 10 mg/L chloramine-T (Tables 1 & 3). A total of 2 of these trials appeared efficacious, while 4 were characterized as inconclusive. Fish species treated included chinook salmon, kokanee salmon, coho salmon, and sockeye salmon.

B. Efficacy at 14.68 mg/L chloramine-T

A total of 2 outbreaks of presumptively diagnosed cases of BGD were treated with 14.68 mg/L chloramine-T (Tables 1 & 3). Both trials involved the tiger musky and 1trial appeared efficacious, while the other trial was characterized as inconclusive.

C. Efficacy at 10 & 15 mg/L chloramine-T

A single preventive treatment of 10 and 15 mg/L chloramine-T (see Table 1) was conducted against external flavobacteriosis. During the course of the study, the treatment dose would vary from 10 mg/L chloramine-T to 15 mg/L chloramine-T. This trial appeared efficacious in controlling mortality in spring chinook salmon.

D. Efficacy at 10, 15, and 20 mg/L chloramine-T

A single treatment of 10, 15, and 20 mg/L chloramine-T was conducted against BGD (see Table 1). During the course of the treatment, the dose was 10 mg/L chloramine-T on day 1 of treatment, 15 mg/L chloramine-T on day 2 of treatment, and 20 mg/L chloramine-T on day 3 of treatment . This trial appeared efficacious in controlling mortality in chinook salmon.

E. Efficacy at 15 mg/L chloramine-T

A total of 28 outbreaks of presumptively diagnosed cases of BGD, external columnaris, and external coldwater disease were treated with 15 mg/L chloramine-T (see Tables 1 & 3). A total of 23 of these trials appeared efficacious, while 5 were characterized as inconclusive. Fish species treated included chinook salmon, chinook salmon, rainbow trout, steelhead trout, brook trout, musky, and tiger musky.

F. Efficacy at 20 mg/L chloramine-T– A total of 39 outbreaks of presumptively diagnosed cases of BGD, external flavobacteriosis, and external columnaris were

treated with 20 mg/L chloramine-T (see Tables 1 - 3). A total of 24 of these trials appeared efficacious, while 5 trials did not appear efficacious, and 10 were characterized as inconclusive. Fish species treated included chinook salmon, chum salmon, rainbow trout, musky, bluegill, and white seabass.

G. Efficacy at 20 & 30 mg/L chloramine-T– A single treatment of 20 & 30 mg/L chloramine-T was conducted against BGD (see Table 1). During the course of the study, the treatment dose would vary from 20 mg/L chloramine-T to 30 mg/L chloramine-T. This trial appeared efficacious in controlling mortality in chinook salmon.

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 10 - 30 mg/L in 78 trials. Fish were treated one, two, or three times on consecutive days for 1 hr. Fourteen different species of fish were treated, and trials involved approximately 19.1 million fish. Treated fish ranged is size from 0.5 - 34.6 in. Water temperature during treatment ranged from 39.4 - 78.0^{o}F, with a mean treatment temperature of 55.9^{o}F. Approximately 68% of trials appeared efficacious, 6% appeared ineffective, and 26% were characterized as inconclusive. Data from the CY 2000 trials support the results of previous Annual Report submissions under INAD #9321 and 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. Control fish were used in only 5 studies (see Study Protocol No. 9321-2000-026, 9321-2000-044, 9321-2000-044(2), 9321-2000-044(3), and 9321-2000-044(4)). 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 #9321 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 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. (ºF)
Whitman Lake Hatchery	1	1.60	CKS	356,579	BGD	3	10	39.4
Cabinet Gorge Hatchery	1	1.23	KOE	10,684,958	BGD	3	10	41.6
Miles City Fish Hatchery	1	0.50	MUH	30,000	BGD	3	14.68	60.0
Dungeness Hatchery	1	34.58	SCS	958	External Flavobacteriosis	11	10 - 15	52.0
Dutchman Creek Fish Farm	4	2.25	GOF	500,000	BGD	1	15	68.0
Mixsawbah SFH	2	3.0 - 3.5	CKS	238,503	BGD	3	15	50.0 - 52.7
Lummi Bay Hatchery	1	2.30	FCS	600,000	BGD	3	15	50.0
Nez Perce Tribal Hatchery	1	4.05	FCS	24,898	BGD	3	15	55.4
Salmon River Hatchery	1	4.50	FCS	369,159	BGD	3	15	62.0
Hackettstown SFH	1	2.30	MUE	8,000	External Columnaris	3	15	68.0
	1	6.00	MUH	16,000	External Columnaris	3	15	68.0
Pequest SFH	5	3.8 - 6.6	RBT	335,500	BGD	3	15	50.0
Bodine SFH	4	1.3 - 2.2	STT	271,508	BGD	3	15	52.0 - 55.0
Mixsawbah SFH	3	1.2 - 2.7	STT	370,688	BGD	3	15	52.0 - 53.3

Table 1. Summary of Year 2000 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. (ºF)
Hackettstown SFH	1	4.40	BLG	15,500	Columnaris	3	20	73.0
Keta Creek Hatchery	3	1.4 - 1.7	CHS	1,567,571	BGD	3	20	46.0 - 48.0
Hackettstown SFH	1	4.00	MUE	6,300	External Columnaris	3	20	68.0
	1	8.48	MUE	6,300	External Columnaris	3	20	68.0
Jones Hatchery	2	5.5 - 5.9	RBT	59,585	BGD	3	20	59.0
	15	8.0 - 10.4	RBT	581,080	BGD	3	20	59.0
	1	14.29	RBT	34,900	BGD	3	20	59.0
Dungeness Hatchery	1	1.50	CKS	7,500	BGD	3	10; 15; 20	41.0
	1	1.65	CKS	32,000	BGD	7	20; 30	45.0

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. (°F)
Jones Hatchery	4	8.7 - 10.2	RBT	189,316	BGD	3	20	59.0
Hubbs Seaworld Research Institute	1	5.29	WSB	3,000	Flavobacteriosis	3	20	69.8

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)
Whitman Lake Hatchery	1	1.60	CKS	356,579	BGD	3	10	39.4
	1	1.38	COS	667,000	BGD	3	10	42.4
Burnett Inlet Hatchery	2	1.21	SOS	828,775	BGD	3	10	42.1 - 43.2
Miles City Fish Hatchery	1	0.50	MUH	30,000	BGD	3	14.68	62.3
Pequest SFH	1	7.14	BKT	50,000	BGD	3	15	50.0
Chelan Hatchery	1	3.80	RBT	195,500	External CWD	3	15	54.0
Bodine SFH	1	1.50	STT	37,578	BGD	3	15	52.0
Mixsawbah SFH	2	1.9 - 2.5	STT	86,011	BGD	3	15	53.0 - 55.0
Hackettstown SFH	1	4.40	BLG	9,100	Columnaris	3	20	78.0
Keta Creek Hatchery	3	1.4 - 1.8	CHS	378,517	BGD	3	20	46.0 - 51.0
Jones Hatchery	6	5.5 - 9.4	RBT	213,167	BGD	3	20	59.0

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

Total Number of Fish Treated:	**19,162,030**
Number of fish treated in efficacious studies	16,117,487
Number of fish treated in non-efficacious studies	192,316
Number of fish treated in inconclusive studies	2,852,227
Total Number of Rearing Units Treated:	**78**
Rearing Units in Efficacious Studies	53
Rearing Units in Non-efficacious Studies	5
Rearing Units in Inconclusive Studies	20

Treatment Regimes and Frequency Used:

10 mg/L - three times	6 trials	14.68 mg/L - three times	2 trials
15 mg/L - one time	4 trials	15 mg/L - three times	24 trials
20 mg/L - three times	39 trials	10 & 15 mg/L - eleven times	1 trial
10, 15, & 20 mg/L - three times	1 trial	20 & 30 mg/L - seven times	1 trial

Treatment Water Temperature (^{o}F):

Temperature Range	39.4 - 78.0
Mean Temperature	55.9

Size of Treated Fish (in.):

Size Range	0.50 - 34.58

Species Treated:

rainbow and steelhead trout (*Oncorhynchus mykiss*)
chinook salmon *(O. tshawytscha)*
sockeye and kokanee salmon (*O. nerka)*
chum salmon (*O. keta)*
brook trout (*Salvelinus fontinalis*)
bluegill (*Lepomis macrochirus*)
goldfish (*Carassius auratus*)
muskie (*Esox masquinongy*)
tiger muskie (*muskellunge x northern pike)*
white seabass (*Atractoscion nobilis*)

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, or BGD like symptoms.

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, or BGD like symptoms, 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.