

Chloramine-T Clinical Field Trials - INAD 4000

Year 2001 Annual Summary Report on the Use of Chloramine-T in Clinical Field Efficacy Trials

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Summary

The efficacy of chloramine-T was evaluated in 74 disease control/prevention trials during calendar year (CY) 2001. Trials were conducted at six U. S. Fish and Wildlife Service (Service) hatcheries and one U. S. Geological Survey (USGS) facility to control mortality caused by the following fish diseases: (1) bacterial gill disease, (2) external columnaris, (3) furunculosis caused by *Aeromonas salmonicida*, or motile aeromonad disease caused by *A. hydrophila*. Fish species treated included: (1) yellow perch *Perca flavescens*, (2) Apache trout *Oncorhynchus apache*, (3) cutthroat trout *O. clarki*, (4) rainbow and steelhead trout *O. mykiss*, and (5) brook trout *Salvelinus fontinalis*. The use of chloramine-T by the Service has been granted by the U. S. Food and Drug Administration 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 efficacy trials conducted during CY2001 involved approximately 5.3 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 two trials conducted with apache trout, chloramine-T treatments were administered four times. Approximately 60% of trials appeared efficacious, 15% appeared ineffective, and 15% 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 U. S. Food and Drug Administration (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 (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 purpose of this report is to summarize the results of CY2001 supplemental chloramine-T field efficacy data. Similar data have been submitted by the Service in previous years. We anticipate that CY2001 data will be used to enhance the existing chloramine-T database established from previous years, and will be considered in the “body of evidence” for the purpose of developing an appropriate label claim for the use of chloramine-T in aquaculture.

Facilities, Materials, Treatment Procedures

1. Facilities

A total of 6 Service National Fish Hatcheries (NFH) and one U. S. Geological Service (USGS) facility used chloramine-T to control/prevent mortality caused by various fish diseases.

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 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,

dissolved chemical added to the rearing unit, and rearing unit contents 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 the following concentrations:

1. 10 mg/L - 1 trial
2. 12 mg/L - 30 trials
3. 15 mg/L - 43 trials.

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, 3, or 4 times to control mortality, and was typically used intermittently to prevent mortality. Intermittent use followed no predetermined treatment schedule, and 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 2000 Trials

1. Species and size of fish treated

The following fish species were treated with chloramine-T during CY 2001:

1. rainbow trout
2. steelhead trout
3. cutthroat trout
4. Apache trout
5. brook trout
6. yellow perch

Approximately 86% of the treated fish were less than 5" in length.

2. Diseases treated

The disease treated most frequently was characterized as BGD. Other diseases treated are listed below:

1. External columnaris caused by *Flavobacterium columnare*
2. Furunculosis caused by *Aeromonas salmonicida*
3. Motile aeromonad diseases caused by *A. hydrophila*.

Data Collected

1. Pathologist's report

Pathologist reports were submitted with approximately 4% of trials in CY 2001.

All submitted pathologist reports were associated with studies conducted at Hagerman NFH. Fish health pathology reports include the following information:

1. A description of how the identity of disease agent(s) was verified
2. Disease identification records that confirm the presence of the disease agent
3. The name and title of the individual performing the diagnosis.

Additionally, pathology reports often provide documentation that there were no secondary infections or infestations caused by unrelated disease agents in a population of test fish. Pathology reports provide essential information if efforts are to expand/extend an existing approved label.

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.

Based on correspondence with FDA, in which technical sections have been completed for the following:

1. Effectiveness of chloramine-T at a concentration of 12 ppm administered as a 60 min bath every other day for three treatments for the control of mortality associated with bacterial gill disease in freshwater-reared salmonids (we refer to your file number INAD 4000 H-0071 dated July 11, 2000).
2. Safety of chloramine-T at a concentration of 20 ppm administered as a 60 min bath on three consecutive or alternate days for the control of mortality associated with bacterial gill disease in freshwater-reared salmonids (we refer to your file number INAD 4000 P-0093 dated September 13, 2002).

As a result of the completed technical sections, mortality data are no longer required if chloramine-T was administered at a dosage of 12 - 20ppm on three alternate or consecutive days to control mortality associated with bacterial gill disease in freshwater-reared salmonids. 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 2001 trials, for the most part, support the findings detailed in the 1995 - 2000 Annual Reports on the Use of Chloramine-T Under INAD #4000.

2. Observations on the efficacy of chloramine-T

A. Efficacy at 10 mg/L chloramine-T

One outbreak of external columnaris in yellow perch was treated with 10mg/L chloramine-T (Table 2). This trial was characterized as unsuccessful.

B. Efficacy at 12 mg/L chloramine-T

A total of 30 outbreaks of presumptively diagnosed BGD in rainbow trout were treated with 12 mg/L chloramine-T (Tables 1-3). A total of 17 (57%) of the 30 trials appeared efficacious, nine (30%) of the trials did not appear efficacious, and four (13%) of the 30 trials were characterized as inconclusive.

C. Efficacy at 15 mg/L chloramine-T

A total of 43 outbreaks of BGD, external columnaris, furunculosis, or motile aeromonad disease were treated with 15 mg/L chloramine-T (Tables 1 - 3). A total of 27 (63%) of the 43 trials appeared to be successful, five (12%) of the 43 trials did not appear to be efficacious, and 11 (25%) of the 43 trials were characterized as inconclusive. Fish species treated included: apache trout, brook trout, cutthroat trout, rainbow trout, steelhead, and yellow perch.

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 - 15 mg/L in 74 trials. Fish were treated one, two, three, or four times on alternate days for 1 hr. Six different species of fish were treated, and trials involved approximately 5.3 million fish. Treated fish ranged in size from 1.0 - 9.1in. Overall mean water temperature during the treatment trials was 54.3°F (range: 48.0 - 68.0°F). Chloramine-T appeared to be effective in controlling mortality in test fish in approximately 60% of trials, appeared ineffective in approximately 20% of the trials, and results appeared inconclusive in approximately 20% of the trials. Data from the CY 2001 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 in a variety of fish species. Also, as reported in previous submissions, treatment efficacy appeared to be highest when chloramine-T dosage was used between 12 - 15 mg/L. Furthermore, investigators reported no evidence of toxicity or adverse effects related to chloramine-T treatment. However, based on a lack of untreated control fish, replication, randomization, etc., it is understood that these data can only be considered as ancillary data. 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 2001 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)
Hotchkiss NFH	16	1.2 - 7.7	RBT	1,435,650	BGD	1	12	56.0
	1	6.79	RBT	26,200	BGD	3	12	56.0
Alchesay-Williams Creek NFH	1	1.64	BKT	131,800	BGD	1	15	51.0
	2	2.4 - 4.2	CUT	62,400	BGD	1	15	51.0
	4	1.6 - 2.4	RBT	127,400	BGD	1	15	51.0
Hotchkiss NFH	3	3.5 - 6.8	RBT	95,978	BGD	1	15	56.0
Alchesay-Williams Creek NFH	1	2.72	APT	33,700	BGD	2	15	51.0
	1	1.69	CUT	39,400	BGD	2	15	51.0
	2	1.0 - 2.0	RBT	421,673	BGD	2	15	51.0
Alchesay-Williams Creek NFH	1	2.45	APT	16,200	BGD	3	15	51.0
	2	1.95	BKT	115,500	BGD	3	15	51.0

Table 1. Summary of Year 2001 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)
Jackson NFH	1	4.75	CUT	140,000	BGD	3	15	48.0
Alchesay-Williams Creek NFH	2	2.0 - 3.7	RBT	308,562	BGD	3	15	51.0 & 67.0
Garrison Dam NFH	1	1.00	RBT	192,000	BGD	3	15	50.0
Norfolk NFH	4	3.4 - 4.0	RBT	427,500	BGD	3	15	50.0 - 54.0
Alchesay-Williams Creek NFH	2	2.0 - 2.4	APT	30,100	BGD	4	15	51.0

Table 2. Summary of Year 2001 Chloramine-T Efficacy Results -Ineffective 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)
Upper Midwest Environmental Sciences Center	1	2.50	YEP	108	External Columnaris	3	10	68.0
Hotchkiss NFH	9	1.6 - 4.4	RBT	647,561	BGD	1	12	56.0
Hotchkiss NFH	1	3.10	RBT	64,648	BGD	1	15	56.0
	1	5.93	RBT	17,843	BGD	2	15	56.0
Alchesay-Williams Creek NFH	2	1.2 - 1.3	APT	91,600	BGD	3	15	51.0
Upper Midwest Environmental Sciences Center	1	2.50	YEP	106	External Columnaris	3	15	68.0

Table 3. Summary of Year 2001 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)
Alchesay-Williams Creek NFH	1	2.10	RBT	17,290	BGD	1	12	51.0
Hotchkiss NFH	2	3.3 - 3.5	RBT	161,500	BGD	1	12	56.0
Alchesay-Williams Creek NFH	1	2.07	RBT	37,529	BGD	2	12	51.0
Alchesay-Williams Creek NFH	1	9.10	APT	26,580	BGD	1	15	50.0
	5	1.6 - 2.6	RBT	121,660	BGD	1	15	51.0
Norfolk NFH	1	3.88	RBT	62,500	BGD	1	15	54.0
Hagerman NFH	1	3.40	STT	86,000	Aeromonas Salmonicida	1	15	59.0
Alchesay-Williams Creek NFH	1	1.88	RBT	25,092	BGD	2	15	51.0
Hagerman NFH	1	7.00	STT	40,000	Aeromonus Hydrophila	3	15	59.0
	1	3.40	STT	151,580	Aeromonas Salmonicida	3	15	59.0

Table 4. Summary Data Regarding Year 2001 Chloramine-T Efficacy Studies

Total Number of Fish Treated:	<u>5,255,660</u>
Number of fish treated in efficacious studies	3,704,063
Number of fish treated in non-efficacious studies	821,866
Number of fish treated in inconclusive studies	729,731
Total Number of Rearing Units Treated:	74
Number of Rearing Units used in Efficacious Studies	44
Number of Rearing Units used in Ineffective Studies	15
Number of Rearing used Units in Inconclusive Studies	15
Treatment Regimes and Frequency Used:	
10 mg/L - three times	1 trial
12 mg/L - one time	28 trials
12 mg/L - two times	1 trial
12 mg/L - three times	1 trial
15 mg/L -one time	19 trials
15 mg/L - two times	6 trials
15 mg/L - three times	16 trials
15 mg/L - four times	2 trials
Treatment Water Temperature (°F):	
Temperature Range	48.0 - 68.0
Mean Temperature	54.3
Size of Treated Fish (in.):	
Size Range	1.0 - 9.1
Species Treated:	apache trout <i>Oncorhynchus apache</i> cutthroat trout <i>O. clarki</i> rainbow trout <i>O. mykiss</i> steelhead trout <i>O. mykiss</i> brook trout <i>Salvelinus fontinalis</i> yellow perch <i>Perca flavescens</i>
