

Chloramine-T Clinical Field Trials - INAD 9321

Year 2003 Annual Summary Report on the Use of Chloramine-T in Clinical Field Efficacy Trials

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Summary

Chloramine-T has been used effectively in the U. S. under compassionate INAD Exemption #9321 to control mortality in a variety of fish caused by common fish bacterial pathogens. In calendar year 2003 the efficacy of chloramine-T (CLT) was evaluated in 52 disease trials involving approximately 4.5 million fish to control mortality in a variety of test fish caused by bacterial gill disease (BGD), external bacterial coldwater disease, or columnaris. Trials were conducted at 13 fish culture facilities, including five U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), seven state fish hatcheries, and one private fish hatchery. The compassionate study protocol under which treatments were administered allowed the investigator to use chloramine-T on either consecutive or alternate days up to three times/wk for 1hr at dosages ranging from 10 - 20 mg/L. Overall results of trials conducted in calendar year 2003 (CY 03) indicated that approximately 44% of the trials appeared efficacious, 4% appeared ineffective, 21% were characterized as inconclusive. In the remaining 31% of the trials, the Investigators did not report mortality data (based on correspondence with FDA, in which technical sections have been completed).

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). 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. These "other" bacteria include *F. aquatile*, *F. psychrophilus*, *F. columnaris*, as well as other flavobacters and aeromonads and pseudomonads. 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).

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.

Purpose of Report

The purpose of this report is to summarize the results of CY 03 supplemental chloramine-T field efficacy data. Similar data have been submitted by the Service in previous years. We anticipate that CY 03 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

Ten fish culture facilities, including five U.S. Fish and Wildlife Service NFH, seven state fish hatcheries, and one private fish hatchery used chloramine-T to control/prevent mortality caused by various fish diseases. Water temperature

during treatments at the various testing facilities ranged from 49.0 - 72.0 °F, with a mean treatment temperature of 56.3°F.

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 one of the following two manufactures: (1) Deerland Chemical, Corp., Littleton, Co. or (2) B.L. Mitchell, Inc., Greenville, MS. During CY 03, a total of 2,131.8 kg of CLT was used.

3. Treatment Methods

Chloramine-T treatments were administered using either flow-through or a standing bath treatment methods. 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 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 the following:

1. 10 mg/L: 3 trials
2. 15 mg/L: 19 trials
3. 20 mg/L: 30 trials

Total 52 trials

5. Number of treatments per disease outbreak

According to the Study Protocol, Investigators were allowed to administer chloramine-T on 1, 2, or 3 consecutive/alternate days when used to control mortality caused by BGD, and once a week when used to prevent mortality. During CY03, CLT was most commonly used on 3 consecutive days to control mortality in fish caused by BGD.

Fish Species Treated and Fish Diseases Involved in CY 03 Trials

1. Species and size of fish treated

Fourteen fish species, including seven salmonids and seven non-salmonids, were treated during CY 03. Treated fish ranged in length from 1.85 - 38.0 in. with a mean length of 6.4 in. Species treated included:

Salmonids: (1) apache trout *Oncorhynchus apache*, (2) brook trout *Salvelinus fontinalis*, (3) cutthroat trout *O. clarki*, (4) lake trout *S. namaycush*, (5) rainbow trout *O. mykiss*, (6) steelhead trout *O. mykiss*, (7) summer chinook salmon *O. tshawytscha*

Non-salmonids: (1) blue catfish *Ictalurus furcatus*, (2) channel catfish *I. punctatus*, (3) lake sturgeon *Acipenser fulvescens*, (4) largemouth bass *Micropterus salmoides*, (5) muskie *Esox masquinongy*, (6) smallmouth bass *M. dolomieu*, (7) walleye *Stizostedion vitreum*.

2. Diseases treated

The disease treated most frequently was characterized as BGD. Other diagnosed diseases included external columnaris and external bacterial coldwater disease

Data Collected

1. Pathologist's report

In CY 03 a pathologist's report was submitted for 31% of the studies. Pathology reports are important for accurate interpretation of study results because they typically contain 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, 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 study Investigators. Such reports were routed through the Study Monitor for review, and then sent to the Bozeman AADAP Office for review, data analysis, data basing, and storage in permanent files.

Based on correspondence with FDA, the following efficacy and safety technical sections have been completed:

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. In all other cases, efforts were made to collect all required mortality data. However, for a variety of reasons, mortality data were not always collected for the entire required data collection 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.

Discussion of Study Results

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

Results of CY 03 trials conducted under Compassionate INAD exemption #9321 are similar to results detailed in reports previous submitted to FDA under INAD's #9321 and #4000.

2. General observations on the efficacy of CLT for the control of bacterial

diseases in salmonid and non-salmonid fish (Note: Table 1 provides a summary of all trials that appeared efficacious; Table 2 provides a summary of all trials that appeared ineffective; Table 3 provides a summary of all inconclusive trials; Table 4 provides a summary of all trials where mortality data was not required; Table 5 provides summary data for all trials; and Tables 6a and 6b provide a brief description of all trials conducted during CY 2003 under INAD #9321; Table 6a lists trials sorted by study number; Table 6b is a list of trials sorted first by disease treated, second by whether treatments were efficacious or not, and lastly by fish species).

A. Efficacy at 10 mg/L chloramine-T

A total of one BGD and two external coldwater disease (CWD) trials were conducted using 10 mg/L chloramine-T (Tables 1 & 3). The trial involving treatment of fish diagnosed with BGD appeared efficacious. One of the two trials involving treatment of fish diagnosed with external CWD appeared efficacious while the other was characterized as inconclusive. Fish species treated with 10 mg/L chloramine-T were cutthroat and steelhead trout.

B. Efficacy at 15 mg/L chloramine-T

A total of 19 trials were conducted with 15 mg/l chloramine-T, including 15 trials with salmonids (species included apache trout, brook trout, lake trout, rainbow trout, and steelhead trout) diagnosed with BGD, one trial with lake sturgeon diagnosed with BGD, two trials with summer chinook salmon diagnosed with

columnaris, and one trials with cutthroat trout diagnosed with external CWD (see Tables 1 - 3). Of the 15 trials in which BGD was diagnosed in salmonids, treatment in 12 (80%) of the trials appeared efficacious, 1 (7%) of the trials did not appear effective, and 2 (13%) of the trials were characterized as inconclusive. One trial that involved lake sturgeon diagnosed with BGD appeared efficacious.

Results from the two trials in which Summer chinook salmon diagnosed with columnaris were treated with 15 mg/L chloramine-T were characterized as inconclusive. Results from the trial in which cutthroat trout diagnosed with external CWD were treated with 15 mg/L chloramine-T were also characterized as inconclusive.

C. Efficacy at 20 mg/L chloramine-T

A total of 30 trials were conducted with 20 mg/L chloramine-T, including 23 trials in which rainbow trout were diagnosed with BGD, one trial in which musky were diagnosed with BGD, and six trials in which blue catfish, channel catfish, lake sturgeon, largemouth bass, smallmouth bass, and walleye were diagnosed with external columnaris (see Tables 1 - 3). Of the 23 trials in which BGD was diagnosed in rainbow trout, 2 (9%) of the trials appeared efficacious, 5 (22%) of the trials were characterized as inconclusive, and 16 (69%) were trials in which mortality collection and reporting were not required. The trial that involved musky diagnosed with BGD appeared efficacious.

Five treatment trials involving channel catfish, lake sturgeon, largemouth bass, smallmouth bass, and walleye diagnosed with columnaris appeared to be efficacious. One trial involving blue catfish diagnosed with columnaris did not appear efficacious.

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 - 20 mg/L in 52 trials. Fish were treated one, two, or three times to control mortality, and once a week when used to prevent mortality. Fourteen different species of fish were treated, and trials involved approximately 4.5 million fish. Treated fish ranged in size from 1.85 - 38.0 in. Water temperature during treatment ranged from 49.0 - 72.0°F, with a mean treatment temperature of 56.3°F. Approximately 44% of trials appeared efficacious, 4% appeared ineffective, 21% were characterized as inconclusive, and in 31% of the trials, mortality data collection and reporting were not required. No evidence of toxicity or adverse effects related to CLT treatment were reported. Data from the CY 03 trials support the results of previous Annual Report submissions under INAD #9321 and INAD #4000 that indicate that chloramine-T treatment is safe and effective to control/prevent mortality in a variety of fish species caused BGD. Control fish were used in only one study (see

Study No. 9321-03-004). As a result of the lack of quality criteria, such as dose verification, use of controls, replicates and randomization, it is understood that these data will 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.

References

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Table 1. Summary of Year 2003 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)
Bozeman FTC	1	2.60	CUT	3,377	BGD	3	10	53.7
Magic Valley Steelhead SFH	1	4.00	STT	180,000	BGD	3	10	58.0
Pequest SFH	1	7.40	BKT	15,000	BGD	1	15	50.0
Alchesay-Williams Creek NFH	1	4.00	APT	31,000	BGD	3	15	50.0
Jordan River NFH	1	3.51	LAT	1,857,714	BGD	3	15	49.0
Pendills Creek NFH	2	3.4 - 3.5	LAT	550,900	BGD	3	15	51.0 - 53.0
Genoa NFH	1	4.00	LST	20,000	BGD	3	15	68.0
Alchesay-Williams Creek NFH	2	2.00	RBT	229,000	BGD	3	15	51.0
Chelan SFH	2	4.4 - 4.8	RBT	108,000	BGD	3	15	56.6 - 58.0
Mixsawbah SFH	1	1.85	RBT	83,600	BGD	3	15	54.5
Pequest SFH	2	6.2 - 6.4	RBT	67,500	BGD	3	15	50.0
Jones Hatchery	1	7.70	RBT	26,000	BGD	2	20	59.0
Genoa NFH	1	2.60	CCF	400	Columnaris	3	20	52.0
	1	7.95	LST	394	Columnaris	3	20	52.0
	1	4.40	LMB	4,490	Columnaris	3	20	52.0

Table 1. Summary of Year 2003 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. (°F)
Hackettstown SFH	1	3.60	MUE	11,000	BGD	3	20	72.0
Genoa NFH	1	3.80	SMB	2,000	Columnaris	3	20	52.0
	1	6.30	WAE	1,681	Columnaris	3	20	52.0
Jones Hatchery	1	6.60	RBT	20,000	BGD	4	20	59.0

Table 2. Summary of Year 2003 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)
Pendills Creek NFH	1	3.21	LAT	148,000	BGD	3	15	52.0
Genoa NFH	1	9.00	BCF	141	Columnaris	3	20	52.0

Table 3. Summary of Year 2003 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)
Hayspur SFH	1	9.00	CUT	2,469	External CWD	3	10	52.0
Chelan SFH	1	4.40	RBT	54,000	BGD	2	15	58.0
Chelan SFH	1	3.20	CUT	87,240	External CWD	3	15	57.0
Alchesay-Williams Creek NFH	1	2.56	RBT	255,251	BGD	3	15	51.0
Eastbank SFH	1	38.00	SUS	454	Columnaris	3	15	56.6
Eastbank SFH	1	38.00	SUS	434	Columnaris	6	15	58.4
Jones Hatchery	4	2.2 - 8.0	RBT	156,000	BGD	1	20	59.0
Jones Hatchery	1	9.10	RBT	33,000	BGD	4	20	59.0

Table 4. Summary of Year 2003 Chloramine-T Efficacy Results -Studies where efficacy data was not needed

Hatchery	Number of trials where mortality was not needed	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Temp. (°F)
Jones Hatchery	16	2.6 - 9.6	RBT	555,000	BGD	3	20	59.0

Table 5. Summary Data Regarding Year 2003 Chloramine-T Efficacy Studies

Total Number of Fish Treated: 4,504,045

Number of fish treated in efficacious studies	3,212,056
Number of fish treated in non-efficacious studies	148,141
Number of fish treated in inconclusive studies	588,848
Number of fish where efficacy was not needed	555,000

Total Number of Studies: 52

Rearing Units in Efficacious Studies	23
Rearing Units in Non-efficacious Studies	2
Rearing Units in Inconclusive Studies	11
Rearing Units where efficacy was not needed	16

Treatment Regimes and Frequency Used:

10 mg/L - three times	3 trials
15 mg/L - one time	1 trial
15 mg/L - two times	1 trial
15 mg/L - three times	16 trials
15 mg/L - six times	1 trial
20 mg/L - one time	4 trials
20 mg/L - two times	1 trial
20 mg/L - three times	23 trials
20 mg/L - four times	2 trials

Treatment Water Temperature (°F):

Temperature Range	49.0 - 72.0
Mean Temperature	56.3

Size of Treated Fish (in.):

Size Range	1.85 - 38.0
Mean Length	6.4

Species Treated:

Salmonids:

apache trout *Oncorhynchus apache*
brook trout *Salvelinus fontinalis*
cutthroat trout *O. clarki*
lake trout *S. namaycush*
rainbow trout *O. mykiss*
steelhead trout *O. mykiss*
summer chinook salmon *O. tshawytscha*

Non-salmonids:

blue catfish *Ictalurus furcatus*
channel catfish *I. punctatus*
lake sturgeon *Acipenser fulvescens*
largemouth bass *Micropterus salmoides*
muskie *Esox masquinongy*
smallmouth bass *M. dolomieu*
walleye *Stizostedion vitreum*
