Chloramine-T Clinical Field Trials - INAD 9321

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 61 disease control/prevention trials during calender year (CY) 2001. Trials were conducted at 11 state hatcheries, 3 private hatcheries, and 3 tribal hatcheries to control mortality caused by the following fish diseases: (1) bacterial gill disease, (2) external columnaris, and (3) external bacterial coldwater disease. Ten different fish species were treated. The U.S. Food and Drug Administration has authorized the use of chloramine-T by the U.S. Fish and Wildlife Service under Compassionate INAD Exemption #9321 for the purpose of collecting ancillary efficacy data to support a new animal drug approval for chloramine-T.

Chloramine-T efficacy trials conducted during CY2001 involved approximately 9.2 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 - 20 mg/L. Approximately 84% of the trials appeared efficacious, 8% appeared ineffective, and 8% 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 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 11 state fish hatcheries, three private hatcheries, and three tribal hatcheries 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. During CY 2001, a total of 706.45 kg of chl-T was used in 61 efficacy 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 the following:

1. 10 mg/L - 7 trials
2. 15 mg/L - 21 trials
3. 20 mg/L - 33 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 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 2001 Trials

1. Species and size of fish treated

The following 11 fish species were treated during CY 2001:

1. rainbow trout *Oncorhynchus mykiss*
2. steelhead trout *O. mykiss*
3. coho salmon *O. kisutch*
4. chinook salmon *O. tshawytscha*
5. chum salmon *O. keta*
6. kokanee salmon *O. nerka*
7. apache trout *O. apache*
8. muskie *Esox masquinongy*
9. northern pike *E. lucius*
10. tiger muskie *muskellunge x northern pike*,
11. bluegill *Lepomis macrochirus*.

Approximately 62% 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 included external columnaris and external bacterial coldwater disease

Data Collected

1. Pathologist's report

In CY 2001a pathologist's report was submitted for 100% of the studies. This is a dramatic improvement over the number of pathologist reports submitted in CY2000, when only 68% of the data submissions included a pathology report. 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. copies of a pathology report or the 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, 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. In all other cases, efforts were made to collect all required mortality data. However, for a variety of reasons, mortality data was not always collected for the entire required 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 CY2001 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. Observations on the efficacy of chloramine-T

A. Efficacy at 10 mg/L chloramine-T

A total of five outbreaks of BGD and two outbreaks of external coldwater disease were treated with 10 mg/L chloramine-T (Tables 1 & 2). All trials involving treatment of chinook and kokanee salmon diagnosed with BGD appeared

efficacious. None of the trials involving treatment of rainbow trout diagnosed with external coldwater disease appeared efficacious.

B. Efficacy at 15 mg/L chloramine-T

A total of 19 outbreaks of BGD and two outbreaks of external coldwater disease were treated with 15 mg/L chloramine-T (see Tables 1 & 3). All trials involving treatment of fish diagnosed with BGD appeared efficacious. One of the two trials involving treatment of fish diagnosed with external coldwater disease appeared efficacious while the other appeared inconclusive. Fish species treated included chinook salmon, coho salmon, rainbow trout, and steelhead trout.

F. Efficacy at 20 mg/L chloramine-T– A total of 25 outbreaks of BGD in salmonids, two outbreaks of BGD in tiger musky, one outbreak each of BGD in musky, northern pike, and bluegill, one outbreak of external columnaris in bluegill, and two outbreaks of external coldwater disease were treated with 20 mg/L chloramine-T (n = 33 trials; see Tables 1 - 3). Of the 25 trials in which BGD was diagnosed in salmonids, 20 (80%) of the trials appeared efficacious, 1 (5%) of the trials did not appear efficacious, and 4 (16%) of the trials were characterized as inconclusive. Salmonid species diagnosed with BGD and treated with 20 mg/L chloramine-T included apache trout, chinook salmon, chum salmon, rainbow trout, and steelhead trout.

Steelhead trout diagnosed with bacterial coldwater disease were treated with 20 mg/L chloramine-T in two trials. Neither treatment trial appeared efficacious.

All five treatment trials involving musky, northern pike, tiger musky, or bluegill diagnosed with BGD appeared efficacious. One trial involving bluegill diagnosed with external columnaris also appeared 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 61 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 9.2 million fish. Treated fish ranged is size from 1.0 - 34.6 in. Water temperature during treatment ranged from 34.7 - 72.0^{o}F, with a mean treatment temperature of 54.8^{o}F. Approximately 84% of trials appeared efficacious, 8% appeared ineffective, and 8% were characterized as inconclusive. Data from the CY 2001 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 in a variety of fish species. Also as reported in previous submissions, treatment efficacy appeared to be highest when chloramine-T dosage was 10-20 mg/L. Furthermore, investigators reported no evidence

of toxicity or adverse effects related to chloramine-T treatment. Control fish were used in only 2 studies (see Study Protocol No. 9321-01-031, and 9321-01-032). 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 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)
Crystal Lake Hatchery	1	1.70	CKS	667,600	BGD	3	10	34.7
Cabinet Gorge Hatchery	4	1.23	KOE	2,250,000	BGD	3	10	45.0
Lummi Bay Hatchery	1	1.80	CKS	375,000	BGD	3	15	45.0
Mixsawbah SFH	3	2.1 - 2.8	CKS	448,250	BGD	3	15	49.4 - 52.0
Tulalip Salmon Hatchery	1	2.10	CKS	1,296,000	BGD	3	15	43.0
Mixsawbah SFH	2	2.3 - 2.5	COS	182,000	BGD	3	15	50.0 - 50.5
Pequest SFH	1	4.10	RBT	45,000	BGD	3	15	50.0
Rangen Research Hatchery	1	1.50	RBT	138,000	BGD	3	15	59.0
Bodine SFH	5	1.6 - 3.0	STT	600,000	BGD	3	15	54.0
Mixsawbah SFH	2	1.6 - 2.4	STT	169,686	BGD	3	15	52.0
Chelan SFH	1	2.29	RBT	74,000	BGD	4	15	53.0
	1	1.40	STT	48,400	BGD	4	15	53.0
	1	3.70	RBT	20,680	BGD	5	15	55.0
Dungeness SFH	1	34.58	CKS	772	External CWD	9	15	52.0
Jones Hatchery	1	5.70	RBT	25,101	BGD	2	20	59.0
Hackettstown SFH	1	4.50	BLG	4,000	BGD	3	20	67.0
	1	4.50	BLG	10,800	External Columnaris	3	20	72.0

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)
Keta Creek Hatchery	2	2.20	CKS	590,000	BGD	3	20	46.0
	1	1.80	CHS	84,200	BGD	3	20	47.0
Hackettstown SFH	1	1.30	MUE	86,000	BGD	3	20	67.0
	1	1.50	NOP	156,900	BGD	3	20	65.0
Jones Hatchery	15	5.6 - 10.6	RBT	542,032	BGD	3	20	59.0
Hackettstown SFH	2	1.6 - 3.8	MUH	54,555	BGD	3	20	67.0 - 68.0
Silver Creek SFH	1	8.00	APT	56,000	BGD	6	20	63.0

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)
Chelan SFH	2	3.04	RBT	174,078	External CWD	3	10	52.0
Jones Hatchery	1	10.80	RBT	33,643	BGD	3	20	59.0
Marblemount SFH	1	1.00	STT	500,000	External CWD	13	20	44.0
Wallace River SFH	1	1.20	STT	160,000	External CWD	17	20	44.0

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)
Tokul Creek Hatchery	1	1.50	STT	240,000	External CWD	14	15	54.0
Jones Hatchery	4	5.3 - 13.3	RBT	131,231	BGD	3	20	59.0

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

Total Number of Fish Treated:	**9,163,928**
Number of fish treated in efficacious studies	7,924,976
Number of fish treated in non-efficacious studies	867,721
Number of fish treated in inconclusive studies	371,231
Total Number of Studies:	**61**
Rearing Units in Efficacious Studies	51
Rearing Units in Non-efficacious Studies	5
Rearing Units in Inconclusive Studies	5

Treatment Regimes and Frequency Used:

10 mg/L - three times	7 trials	15 mg/L - three times	16 trials
15 mg/L - four times	2 trials	15 mg/L - five times	1 trial
15 mg/L - nine times	1 trial	20 mg/L - two times	1 trial
20 mg/L - three times	29 trials	20 mg/L - six times	1 trial
20 mg/L - thirteen times	1 trial	20 mg/L - fourteen times	1 trial
20 mg/L - seventeen times	1 trial		

Treatment Water Temperature (^{o}F):

Temperature Range	34.7 - 72.0
Mean Temperature	54.8

Size of Treated Fish (in.):

Size Range	1.00 - 34.58

Species Treated:

chinook salmon *(O. tshawytscha)*
chum salmon (*O. keta)*
apache trout (*O. apache*)
muskie (*Esox masquinongy*)
tiger muskie (*muskellunge x northern pike)*
rainbow and steelhead trout (*Oncorhynchus mykiss*)
kokanee salmon (*O. nerka)*
coho salmon (*O. kisutch*)
bluegill (*Lepomis macrochirus*)
northern pike (*E.lucius*)