Diquat Clinical Field Trials - INAD #10-969

Year 2005 Annual Summary Report on the Use of Diquat in Clinical Field Efficacy Trials

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Summary

Diquat has been used effectively in the U. S. under compassionate INAD Exemption #10-969 to control mortality in a variety of fish species caused by common external fish bacterial pathogens. In calendar year 2005 (CY05) the efficacy of diquat (DQT) was evaluated in 38 disease trials involving approximately 3.0 million fish to control mortality in a variety of fish species caused by columnaris or bacterial gill disease. Trials were conducted at four fish culture facilities, including one U.S. Fish and Wildlife Service (Service) National Fish Hatchery (NFH) and three state fish hatcheries. Use of DQT under Protocol #10-969 allowed the investigator to administer therapeutic dosages of DQT to treat sick fish using one of the following two treatment options: 1) 2 - 18 mg/L for 1 - 4 h up to four times on consecutive or alternate days ; or 2) 19 - 28 mg/L for 0.5 - 1 h up to three times on consecutive days. Overall, results indicated that treatments appeared effective in approximately 92% of the trials, ineffective in 3% of the trials, and 5% of the trials were characterized as inconclusive.

Introduction

Diseases of cultured fish often leads to severe losses of fish which can ultimately impact fish stocking programs and commercial fish farms. Such diseases can be caused by infections from a variety of fish pathogens. However, a few of these diseases, including bacterial gill disease (BGD), columnaris, and coldwater disease (CWD), appear to be the most prevalent.

Bacterial gill disease is one of the most serious diseases of intensively cultured fish, particularly young salmonids. If BGD is not diagnosed and treated early, significant mortality may occur in a very short period of time. Fish mortality is generally not a direct result of the infection, but is a consequence of the infection (i.e., structural changes in gill morphology). Stressors associated with intense fish culture may predispose fish to such an infection. 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 result in 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).

Columnaris - 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 pathogens such as *F. aquatile, F. psychrophilus* (causative agent of CWD), and *F. columnare* (causative agent of columnaris). Columnaris disease has been reported to cause significant mortality in a wide variety of fish (Post 1987), and is particularly devastating to cool and warm water species. Although the optimum temperature for the occurrence of columnaris disease is approximately 28 - 30^{o}C, epizootics often occur in cultured fishes at 10 - 17^{o}C. *F. columnare* typically first invades the skin of the head region, including the mouth, lips, cheeks, and gills and can result in necrosis of gill tissue. The pathogen also invades injuries or open wounds on the body of the fish. The type of lesions vary with the species of fish (Post 1987). Although *F. columnare* can routinely be detected externally in moribund fish when specimens are collected from the gills or open wounds of infected fish, the pathogen can also be cultured from kidney tissue of seriously infected fish. In such cases, columnaris disease is usually terminal within a relatively short time following bacteriemia (Post 1987).

Coldwater disease occurs most often in coldwater fish species at environmental temperatures between 4 and 10^{o}C (Post 1987). *F. psychrophilus* may invade fins, causing necrosis of tissue, but will also become systemic. Once the presence of the pathogen can be detected from internal organs such as kidney tissue, fish mortality is likely to increase. Hence, it is important to promptly diagnose, preferably before pathogens can be detected in internal tissues (i.e., while the “disease” is still external”), and treat such infections.

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) and other external flavobacteria. However, none of these chemicals have been approved by the FDA to control mortality in freshwater fish caused by such diseases. Although use of such chemicals does not guarantee success, INAD records support the use of chloramine-T and DQT to effectively control mortality in fish caused by external fish bacteria. The success of DQT as a chemotherapeutant that effectively controls mortality caused by external flavobacteria has been attributed to its characterization as a non-selective sanitizing agent that effectively cleans up external fish surfaces, including skin and gills infested with bacteria. This report summarizes use of DQT to control mortality in fish diagnosed with external bacterial diseases when used under INAD #10-969 during CY05.

Purpose of Report

The purpose of this report is to summarize the results of DQT field efficacy trials conducted under INAD #10-969 during CY05. We anticipate that data generated in these trials will be used to enhance data in the existing DQT 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 DQT in aquaculture.

Facilities, Materials, Treatment Procedures

1. Facilities

A total of four fish culture facilities, including one U. S. Fish and Wildlife Service National Fish Hatchery and three state fish hatcheries, used DQT to control mortality in fish caused by variety of fish diseases. Mean water temperature during all treatments was 71.7 °F, and water temperature for individual trials during treatments at the various testing facilities ranged from 49.5 - 78.8 °F.

2. Chemical material

REWARD® (a liquid DQT concentrate supplied by Syngenta Crop Protection, Inc., Greensboro, NC; 37.3% diquat bromide and 62.7% inerts) was the only brand of DQT used in CY05 trials, and remains the only brand of DQT that is allowed to be used under INAD #10-969 . This over-the-counter product contains 2 pounds diquat cation/gal as 3.73 pounds salt/gal.

3. Treatment Methods

Diquat treatments were administered by either a flow-through or standing bath procedure. Both procedures require accurately measured amounts of liquid DQT that need to be pre-mixed in an appropriate amount of non-chlorinated water before administration.

Flow-through procedure - The fully dissolved pre-mixed chemical is metered into rearing units at a rate sufficient to achieve the desired target treatment dose over a period extending from 0.5 - 4 hr period.

Standing bath procedure - Water flow to the rearing unit is turned off, the pre-mixed chemical is added to the rearing unit, and contents of the rearing unit thoroughly mixed to ensure uniform DQT concentration throughout the rearing unit. Thorough mixing is essential to ensure there are no DQT "hot spots" and that regardless of a fishes position in the rearing unit, it is exposed to the target DQT dose. After the 0.5 - 4 hr treatment, water flow is turned on again to flush all DQT from of the rearing unit.

4. Drug dosages

Diquat was used by Investigators at one or both of the following dosage regimens:

1. 2 - 18 mg/L for 1 - 4 hr
2. 19 - 28 mg/L for 1 hr

5. Number of DQT treatments administered per disease outbreak

According to the Study Protocol, Investigators were allowed to administer DQT on (1) 1 - 4 consecutive/alternating days when used at a dose of 2 - 18 mg/L (approximately 55% of trials were conducted using this treatment regimen).

However, the treatment regimen administered in the remaining trials (approximately 45% of the trials) deviated from the protocol. In these trials, fish were treated at a dose of 10 - 24 mg/L DQT for durations that extended from 5 - 49 days. The Investigators noted that the deviations occurred due primarily to past use that supported the fact that high fish losses would results if DQT was not administered for periods exceeding what was allowed in the protocol. In addition, it has been shown that presence of high amounts of fine particles (e.g., uneaten feed, suspended organic solids) can irritate fish gills predisposing fish to columnaris and BGD infections. Such an event is typically associated with a period during the early grow-out phase when fish are fed fine particle sized feed, or when water supplying the hatchery is only coarsely filtered and is not disinfected with either ultraviolet light or ozone.

Fish species that were used in efficacy trials in which these deviations occurred included: walleye, northern pike, musky, largemouth bass, and channel catfish. However, it should be noted that because fish were treated at an early life-stage and will not be available for human consumption for at least 1-3 years, that there should be no concern regarding the withdrawal period.

Fish Species Treated and Fish Diseases Involved in CY05 Trials

1. Species and size of fish treated

A total of six different fish species were treated during CY05, including one salmonid and five non-salmonid fish species. Mean length of treated fish was 3.5 in, and fish size ranged in length from 0.5 - 13.1 in.

Species treated included:

1. fall chinook salmon *Oncorhychus tshawytscha*
2. channel catfish *Ictalurus punctatus*
3. largemouth bass *Micropterus salmoides*
4. muskellunge *Esox masquinongy*
5. northern pike *E. lucius*
6. walleye *Stizostedion vitreum*

2. Diseases treated

The fish disease treated most frequently was characterized as (external) columnaris, which was treated for in 35 (92%) of the 38 trials; and BGD, which was treated for in three (8%) trials.

Data Collected

1. Pathologist's report

In the protocol, there is a request that a fish health biologist or qualified fishery biologist examine moribund and dead fish to try to determine the cause of death, and attach the fish health pathology report to the INAD data packet submitted to the AADAP Office following treatment. Fish health pathology reports can provide confirmation that there was a presumptive or definitive disease diagnosis for which treatment was recommended. Pathology reports were submitted for 68% of the trials submitted in CY05.

2. Mortality data

As stated in the Study Protocol, mortality data was to be collected for at least five days prior to treatment, during treatment, and for at least 14 d post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, for a variety of reasons, not all requested mortality data was collected. Reasons for an incomplete mortality record include : 1) splitting fish into additional rearing units to ease crowding and improve culture conditions, and 2) stocking early life stage fish shortly after final treatment.

Study Results - Discussion

1. General observations on the efficacy of DQT for the control of bacterial diseases in salmonid and non-salmonid fish (Note: Table 1 provides a summary of all trials in which DQT treatments appeared effective; Table 2 provides a summary of all trials in which treatments were ineffective; Table 3 provides a summary of all trials in which treatments appeared inconclusive; Table 4 provides a summary of all treatment trials, including number of trials, number of fish treated, and treatment regimens used; and Tables 5a (trials sorted by study number) and 5b (trials sorted first by disease treated, second by whether or not treatments were effective, and lastly by fish species treated) provide a summary of all trials conducted during CY05 under INAD #10-969).

A. Efficacy of DQT When Used to Treat Columnaris

A total of 35 trials were conducted in which fish diagnosed with columnaris were treated with DQT at doses that ranged from 10 to 24 mg/L DQT for durations that ranged from 1 to 4 h. Fish were treated over a period that extended from 1 to 49 days (see Tables 1 - 3). Below is a list of the fish species that were treated for columnaris and the number of trials conducted using the specified treatment regimens:

channel catfish

1. Dose: 10 mg/L; Duration: 4 h; Treatment period: 2 - 4 days (6 trials)
2. Doses: 10 & 12 mg/L; Duration: 4 h; Treatment period: 6 - 31 days (4 trials)

3. Doses: 10, 12, & 15 mg/L; Duration: 1 h; Treatment period: 2 - 5 days (1 trial)
4. Dose: 12 mg/L; Duration: 4 h; Treatment period: 2 - 5 days (2 trials)
5. Doses: 12 & 18 mg/L: Duration: 1 h; Treatment period: 4 days (1 trial)
6. Doses: 15 & 18 mg/L; Duration: 4 h; Treatment period: 7 & 17 days (1 trial)

largemouth bass

1. Doses: 10 & 12 mg/L; Duration: 4 h; Treatment period: 10 days (1 trial)
2. Doses: 15 & 18 mg/L; Duration: 1 h; Treatment period: 1 - 7 days (1 trial)
3. Dose: 18 mg/L; Duration: 4 h; Treatment period: 1 day (1 trial)

musky

1. Dose: 10 mg/L; Duration: 4 h; Treatment period: 2 days (1 trial)

walleye

1. Dose: 10 mg/L: Duration: 1 - 4 h: Treatment period: 2 - 49 days (7 trials)
2. Dose: 12 mg/L; Duration: 4 h; Treatment period: 3 days (1 trial)
3. Doses: 12, 15 & 18 mg/L; Duration: 1 h; Treatment period: 1 - 15 days (1 trial)
4. Dose: 15 mg/L; Duration: 1 - 2 h; Treatment period: 3 - 4 days (2 trials)
5. Doses: 15 & 18 mg/L; Duration: 2 h; Treatment period: 8 - 15 days (1 trial)
6. Dose: 18 mg/L; Duration: 2 h; Treatment period: 2 - 6 days (3 trials)
7. Doses: 18 & 24 mg/L; Duration: 1 - 2 h; Treatment period: 20 - 21 days (1 trial)

Results from 33 of the above-described trials indicated that treatments appeared effective, while one trial involving walleye was ineffective, and another trial involving walleye was characterized as inconclusive.

B. Efficacy of DQT When Used to Treat Bacterial Gill Disease

A total of three trials were conducted in which fish were diagnosed with BGD and were treated with DQT at doses that ranged from 10 to 15 mg/L for a 1 h duration. Fish were treated over a period that extended from 2 to 25 days (see Tables 1 & 3). Below is a list of the fish species that were treated for BGD, and the number of trials conducted using the specified treatment regimens:

<u>fall chinook salmon</u>

1. Dose: 15 mg/L; Duration:1 h; Treatment period: 2 days (1 trial)

<u>musky and northern pike</u>

1. Dose: 10 mg/L; Duration: 1 h; Treatment period: 11 - 25 days (2 trials)

Results from all of the above-described trials indicated that treatments appeared effective in the two trials involving musky and northern pike, while the trial involving fall chinook salmon was characterized as inconclusive.

3. Observed Toxicity

No toxicity or adverse effects relating to DQT treatments were reported.

Summary of Study Results

Diquat was used at doses ranging from 10 to 24 mg/L in 38 trials to control mortality in a variety of fish species caused by either columnaris or BGD. Fish were treated 1 - 49 times on consecutive or alternate days for durations that ranged from 1 to 4 h. Treatments were administered to six different fish species, and treatment trials involved approximately 3.0 million fish. Mean length of fish treated during CY05 was 3.5 in (range, 0.5 - 13.1 in), and mean water temperature of all trials was 71.7^{o}F (range, 49.5 - 78.8^{o}F). Results from approximately 92% of trials indicated that DQT treatments appeared effective in controlling mortality, ineffective in 3% of the trials, and 5% of the trials were characterized as inconclusive. Investigators reported no evidence of toxicity or adverse effects related to DQT treatment. Although data from these trials will be considered ancillary, trial results should provide useful corroborative data to support a future label claim for DQT. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-969. In future trials conducted under INAD #10-969, efforts will be directed towards the generation of higher quality data and stricter adherence to the study protocol with respect to treatment regimens administered to control mortality in fish caused by external flavobacterial diseases such as BGD and columnaris.

References

Bullock, G.L. 1990, Bacterial gill disease of freshwater fishes, Fish Disease Leaflet 84, U.S. Dept. of the Interior, Fish and Wildlife Service, Washington DC.

Ferguson, H.W., V.E. Ostland, P. Byrne, and J.S. Lumsden. 1991. Experimental production of bacterial gill disease in trout by horizontal transmission and bath challenge. Journal of Aquatic Animal Health 3:118-123.

Post, G.W. 1987. Textbook of fish health. Revised and expanded edition. TFH Publications, Inc., Ltd., Neptune City, New Jersey. 288 pp.

Wakabayashi, H, G.J. Huh and N. Kimura. 1989. Flavobacterium branchiophila sp. now., a causative agent of bacterial gill disease of freshwater fishes. International Journal of Systematic Bacteriology 39:213-216

Table 1. Summary of CY05 Diquat Field Efficacy Trial Results - Effective Treatments

Hatchery	Number of efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (ºF)
Rathbun SFH	6	1.6 - 6.2	CCF	317,779	Columnaris	2 - 4	10	4	55.0 - 77.0
	1	13.05	MUE	1,888	Columnaris	2	10	4	53.5
Spirit Lake SFH	1	0.50	MUE	152,327	BGD	25	10	1	66.5
	1	0.50	NOP	334,056	BGD	11 - 14	10	1	58.9
Rathbun SFH	5	3.3 - 3.5	WAE	152,949	Columnaris	2 - 4	10	4	75.5 - 76.3
Spirit Lake SFH	1	2.30	WAE	32,444	Columnaris	49	10	1	72.6
Rathbun SFH	3	2.8 - 3.0	CCF	253,473	Columnaris	27 - 31	10 & 12	4	76.6 - 78.0
	1	9.10	CCF	9,112	Columnaris	6	10 & 12	4	77.5
	1	2.90	LMB	25,092	Columnaris	10	10 & 12	4	78.5
Rathbun SFH	1	1.00	CCF	322,450	Columnaris	2 - 5	10; 12; 15	1	65.0
Rathbun SFH	2	4.2 - 5.7	CCF	30,804	Columnaris	2 - 5	12	4	63.5 - 78.8
	1	9.30	WAE	21,271	Columnaris	3	12	4	62.0
Rathbun SFH	1	1.10	CCF	212,871	Columnaris	4	12 & 18	1	72.0
Rathbun SFH	1	1.80	WAE	462,011	Columnaris	1 - 15	12; 15; 18	1	75.0
Rathbun Research	1	1.10	WAE	21,000	Columnaris	3	15	2	67.4
Rathbun SFH	1	1.69	CCF	27,781	Columnaris	7 & 17	15 & 18	4	77.0
	1	1.30	LMB	97,912	Columnaris	1 - 7	15 & 18	1	77.0
Rathbun Research	1	2.35	WAE	42,000	Columnaris	8 - 15	15 & 18	2	72.5

Table 1. Summary of CY05 Diquat Field Efficacy Trial Results - Effective Treatments - continued

Hatchery	Number of efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun SFH	1	5.10	LMB	5,217	Columnaris	1	18	4	76.0
Rathbun Research	3	3.1 - 6.0	WAE	24,606	Columnaris	2 - 6	18	2	74.6 - 78.1
Rathbun Research	1	4.30	WAE	4,725	Columnaris	20 - 21	18 & 24	1 - 2 hr	74.0

Table 2. Summary of CY05 Diquat Field Efficacy Trial Results - Ineffective Treatments

Hatchery	Number of ineffective trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun Research	1	1.10	WAE	15,024	Columnaris	4	15	1	66.6

Table 3. Summary of CY05 Diquat Field Efficacy Trial Results - Inconclusive Treatments

Hatchery	Number of inconclusive trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun SFH	1	3.30	WAE	46,941	Columnaris	2	10	4	75.5
Spring Creek NFH	1	3.76	FCS	348,659	BGD	2	15	1	49.5

Table 4. Summary of Number of Treated Fish, Number of Treatment Trials, Treatment Regimens Used, and Fish Species Treated during CY05 Diquat-T Field Efficacy Trials

Total Number of Fish Treated:		**2,962,392**
Number of fish treated in effective trials	2,551,768	
Number of fish treated in ineffective trials	15,024	
Number of fish treated in inconclusive trials	395,600	
Total Number of Trials:		**38**
Number of trials in which treatments were effective		35
Number of trials in which treatments were ineffective		1
Number of trials in which treatment results were Inconclusive		2

Treatment Regimes and Frequency Used:

2 - 18 mg/L for 1 - 4hr; 1 - 49 days	37 trials
18 & 24 mg/L for 1-2hr; 20 - 21days	1 trial

Treatment Water Temperature (^{o}F):

Temperature Range	49.5 - 78.8
Mean Temperature	71.7

Size of Treated Fish (in.):

Fish Size Range	0.5 - 13.1
Mean Fish Size	3.5

Species Treated:

fall chinook salmon *Oncorhychus tshawytscha*
channel catfish *Ictalurus punctatus*
largemouth bass *Micropterus salmoides*
muskellunge *Esox masquinongy*
northern pike *E. lucius*
walleye *Stizostedion vitreum*