Diquat Clinical Field Trials - INAD #10-969

Year 2004 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 2004 (CY04) the efficacy of diquat (DQT) was evaluated in 54 disease trials involving approximately 3.2 million fish to control mortality in a variety of fish species caused by several fish diseases, including columnaris, bacterial gill disease, and bacterial coldwater disease. Trials were conducted at seven fish culture facilities, including one U.S. Fish and Wildlife Service (Service) National Fish Hatchery (NFH) and six 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 - 4hr up to four times on consecutive or alternate days ; or 2) 19 - 28 mg/L for 0.5 - 1hr up to three times on consecutive days. Overall, results indicated that treatments appeared effective in approximately 96% of the trials, while 4% of the trials were characterized as inconclusive.

Introduction

Diseases of cultured fish can result in severe losses of fish, which can then negatively impact fish stocking programs and commercial fish farms. Although there are a number of bacterial infections that can result in substantial fish losses, some of the more prevalent diseases that effect commonly reared fish include bacterial gill disease (BGD), columnaris, and coldwater disease (CWD). 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 within a 24-hr period. Fish mortality is generally not a direct result of the infection, but is a consequence of the infection. 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).

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 samples 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 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 the 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 CY04.

Purpose of Report

The purpose of this report is to summarize the results of DQT field efficacy trials conducted under INAD #10-969 during CY04. 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 seven fish culture facilities, including one Service NFH and six state fish hatcheries, used DQT to control mortality in fish caused by various fish diseases.

Mean water temperature during all treatments was 69.7 °F, and water temperature for individual trials during treatments at the various testing facilities ranged from 45.0 - 78.0 °F.

2. Chemical material

REWARD® (a liquid DQT concentrate supplied by Syngenta Crop Protection, Inc., Greensboro, NC) was the only brand of DQT used in CY04 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 weighed amounts of liquid DQT to be pre-mixed in an appropriate amount of non-chlorinated water before it is administered.

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 35% of trials were conducted using this treatment regimen) or (2) 1 - 3 consecutive days when used at a dose of 19 - 28 mg/L (approximately 6% of trials were conducted using this treatment regimen). However, the treatment regimen administered in the remaining trials (approximately 59% of the trials) deviated from the protocol. In these trials, fish were treated at a dose of 10 - 18 mg/L DQT for durations that extended from 4 - 42 days. The Investigators noted that the deviations occurred due primarily to past use that supported the fact that high fish losses would be occur at fish culture facilities that did not administer

DQT for period exceeding what was allowed in the protocol. In addition, it has been shown that presence of high amounts of fine particles, that irritate fish gills can lead to high susceptibility of fish to columnaris and BGD, may result from either (1) the period that fish are converting to commercial feed and during the early grow-out phase (when fish are fed fine particle sized feed), or (2) when water supplying the hatchery is only coarsely filtered and is not disinfected with either ultraviolet or ozone. Fish species that were used in efficacy trials in which these deviations occurred included: walleye, northern pike, musky and channel catfish. However, it should be noted that because fish were treated at an early life-stage, they were not available for human consumption for at least 1-3 years.

Fish Species Treated and Fish Diseases Involved in CY04 Trials

1. Species and size of fish treated

A total of six different fish species were treated during CY04, including two salmonid and four non-salmonid fish species. Mean length of treated fish was 3.5 in, and fish size ranged in length from 0.5 - 16.0 in.

Species treated included:

1. rainbow trout *Oncorhynchus mykiss*
2. coho salmon *O. kisutch*
3. channel catfish *Ictalurus punctatus*

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 46 (85%) of the 54 trials. Other fish diseases treated included BGD, which was treated for in six (11%) trials, and CWD, which was treated for in two (4%) 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 send the fish health pathology report in with the rest of the INAD data record paperwork. Fish health pathology reports can provide confirmation that there was a definitive disease diagnosis for which treatment was recommended. Unfortunately, pathology reports were submitted for only 13% of the trials submitted in CY04.

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 appeared inconclusive; Table 3 provides a summary of all treatment trials, including number of trials, number of fish treated, and treatment regimens used; and Tables 4a (trials sorted by study number) and 4b (trials sorted first by disease treated, second by whether or note treatments were effective, and lastly by fish species treated) provide a summary of all trials conducted during CY04 under INAD #10-969).

A. Efficacy of DQT When Used to Treat Columnaris

A total of 46 trials were conducted in which fish diagnosed with columnaris were treated with DQT at dosages that ranged from 10 to 18 mg/L DQT for durations

that ranged from 1 to 4hrs. Fish were treated over a period that extended from 2 to 24 days (see Table 1). 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: 4hrs; Treatment period: 2 - 21 days (8 trials)
2. Dose: 12 mg/L; Duration: 1hr; Treatment period: 3 days (1 trial)
3. Dose: 15 mg/L: Duration: 4hrs; Treatment period: 3 - 4 days (4 trials)
4. Dose: 18 mg/L: Duration: 1hr; Treatment period: 3 days (1 trial)
5. Doses:12, 15 & 28 mg/L; Duration: 1 - 2hrs; Treatment period: 4 - 18 days (1 trial)

walleye

1. Dose: 12 mg/L: Duration: 2hrs: Treatment period: 3 days (1 trial)
2. Dose: 15 mg/L; Duration: 4hrs; Treatment period: 2 - 3 days (5 trials)
3. Dose: 18 mg/L; Duration: 2hrs; Treatment period: 7 - 24 days (5 trials)
4. Doses: 12, 15 & 18 mg/L; Duration: 1 - 2hrs; Treatment period: 12 - 21 days (11 trials)
5. Doses: 15 & 18 mg/L; Duration: 1 - 2hrs; Treatment period: 13 - 22 days (9 trials)

Results from all of the above-described trials indicated that treatments appeared effective.

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

A total of six trials were conducted in which fish were diagnosed with BGD were treated with DQT at dosages that ranged from 2 to 20 mg/L for a 1 hr duration. Fish were treated over a period that extended from 2 to 42 days (see Table 1). Below is a list of the fish species that were treated for BGD, and the number of trials conducted using the specified treatment regimens:

rainbow trout

1. Dose: 2 mg/L; Duration:1 hr; Treatment period: 2 days (1 trial)
2. Dose: 20 mg/L; Duration:1 hr; Treatment period: 3 days (1 trial)

musky, northern pike and walleye

1. Dose: 10 mg/L; Duration: 1hr; Treatment period: 10 - 42 days (4 trials)

Results from all of the above-described trials indicated that treatments appeared effective.

C. Efficacy of DQT When Used to Treat CWD

A total of two trials were conducted in which coho salmon were diagnosed with CWD and treated with 20 - 25 mg/L DQT for 1 hr on 3 days (see Table 2). Results from these trials were characterized as inconclusive due to simultaneous treatments of other medications or incomplete collection of the mortality data.

3. Observed Toxicity

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

Summary of Study Results

Diquat was used at dosages ranging from 2 to 25 mg/L in 54 trials to control mortality in a variety of fish species caused by either columnaris, BGD, or CWD. Fish were treated 2 - 42 times on consecutive or alternate days for durations that ranged from 1 to 4 hr. Treatments were administered to six different fish species, and treatment trials involved approximately 3.2 million fish. Mean length of fish treated during CY04 was 3.5 in (range, 0.5 - 16.0 in), and mean water temperature of all trials was 69.7^{o}F (range, 45 - 78^{o}F). Results from approximately 96% of trials indicated that DQT treatments appeared effective in controlling, while 4% were characterized as inconclusive due to simultaneous drug treatments or incomplete collection of the mortality data. 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, columnaris, and CWD.

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 CY04 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)
Erwin NFH	1	16.00	RBT	12,330	BGD	2	2	1	55.0
Rathbun SFH	6	3.4 - 12.7	CCF	315,617	Columnaris	2 - 3	10	4	64.3 - 74.2
	2	2.0 - 2.9	CCF	283,377	Columnaris	8 - 21	10	4	72.1 - 76.0
Spirit Lake SFH	1	0.50	MUE	124,589	BGD	33 - 42	10	1	69.8
	2	0.50	NOP	191,822	BGD	10 - 11	10	1	65.5 - 65.7
	1	2.20	WAE	66,584	BGD	16	10	1	71.5
Rathbun SFH	1	1.38	CCF	250,793	Columnaris	3	12	1	66.0
Rathbun Research	1	1.60	WAE	13,632	Columnaris	3	12	2	68.0
Rathbun SFH	1	1.30	CCF	388,957	Columnaris	4 - 18	12, 15, 18	1 - 2	75.0
Rathbun SFH	10	2.25	WAE	240,000	Columnaris	14 - 16	12, 15, 18	1	71.0
Rathbun Research	1	2.35	WAE	12,258	Columnaris	12 - 21	12, 15, 18	2	72.2
Rathbun Research	4	2.7 - 7.2	CCF	212,522	Columnaris	3 - 4	15	4	72.7 - 78.0
Rathbun Research	5	3.3 - 3.9	WAE	147,285	Columnaris	2 - 3	15	4	71.3 - 73.0
Rathbun SFH	5	2.25	WAE	90,000	Columnaris	14 - 16	15 & 18	1	71.0
Rathbun Research	4	2.35	WAE	59,787	Columnaris	13 - 22	15 &18	2	73.0
Rathbun SFH	1	1.78	CCF	32,786	Columnaris	3	18	1	72.0
Rathbun Research	5	2.4 - 7.8	WAE	28,537	Columnaris	7 - 24	18	2	72.2 - 73.0
Bellingham SFH	1	10.50	RBT	7,584	BGD	3	20	1	45.0

Table 2. Summary of CY04 Diquat Field Efficacy Trial Results - Inconclusive 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. (°F)
Kendall Creek SFH	1	0.75	COS	300,000	CWD	3	20	1	47.0
Wallace River SFH	1	1.78	COS	434,000	CWD	3	25	1	47.0

Table 3. Summary of Number of Treated Fish, Number of Treatment Trials, Treatment Regimens Used, and Fish Species Treated during CY04 Diquat-T Field Efficacy Trials

Total Number of Fish Treated:		**3,212,460**
Number of fish treated in effective trials	2,478,460	
Number of fish treated in inconclusive trials	734,000	
Total Number of Trials:		**54**
Number of trials in which treatments were effective		52
Number of trials in which treatment results were Inconclusive		2

Treatment Regimes and Frequency Used:

2 - 18 mg/L for 1 - 4hr; 1 - 42 days	30 trials
20 - 25 mg/L for 1hr; 3 days	3 trials
12, 15 & 18 mg/L for 1 - 2hr; 4 - 21 days	12 trials
15 & 18 mg/L for 1 - 2hr; 14 -22 days	9 trials

Treatment Water Temperature (°F):

Temperature Range	45.0 - 78.0
Mean Temperature	69.7

Size of Treated Fish (in.):

Fish Size Range	0.5 - 16.0
Mean Fish Size	3.5

Species Treated:

rainbow trout *Oncorhynchus mykiss*
coho salmon *O. kisutch*
channel catfish *Ictalurus punctatus*
muskellunge *Esox masquinongy*
northern pike *E. lucius*
walleye *Stizostedion vitreum*