Diquat (Reward®) Clinical Field Trials - INAD #10-969

Year 2008 Annual Summary Report on the Use of Diquat (Reward®) in Clinical Field Efficacy Trials

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Summary

Diquat (Reward®) 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 2008 (CY08) the efficacy of diquat (DQT) was evaluated in 20 disease trials involving approximately 3.8 million fish to control mortality in a variety of fish species caused by external columnaris or bacterial gill disease. Trials were conducted at four state fish culture facilities. 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 90% of the trials while 10% 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, 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).

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 the use of DQT to control mortality in fish diagnosed with external bacterial diseases when used under INAD #10-969 during CY08.

Purpose of Report

The purpose of this report is to summarize the results of DQT field efficacy trials conducted under INAD #10-969 during CY08. 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 state fish culture facilities used DQT to control mortality in fish caused by BGD or external columnaris. Mean water temperature during all treatments was 69.8 °F, and water temperature for individual trials during treatments at the various testing facilities ranged from 51.0 - 79.3 °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 CY08 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 (Reward®) treatments were administered using either a flow-through or standing bath treatment method. Both procedures called for accurately measured amounts of liquid DQT to be pre-mixed in an appropriate amount of non-chlorinated water before administration. When using a flow-through system, the pre-mixed chemical was metered into rearing units at a rate to achieve the desired treatment concentration during a 1 - 4 h period. When using a standing bath method, water flow to the rearing unit was turned off and the pre-mixed chemical added to the rearing unit and mixed thoroughly to ensure uniform DQT concentration throughout the tank. Thorough mixing was essential to ensure there were no DQT "hot spots." After the treatment, water flow was turned on again to flush the chemical out of the rearing unit.

4. Drug dosages

Diquat (Reward®) was used by Investigators at one or both of the following dosage regimens:

1. 4 - 18 mg/L for 1 hr - 4 hr
2. 20 - 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 or

(2) 1 - 3 times on consecutive days at a dosage of 19 - 28 mg/L (approximately 40% of trials were conducted using these treatment regimens).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 60% of the trials) deviated from the protocol. In these trials, fish were treated at a dose of 10 - 28 mg/L DQT for durations that extended from 5 - 58 days. The Investigators noted that the deviations occurred primarily due to past use that supported the fact that high fish losses would result 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: kokanee salmon, walleye, northern pike, muskellunge, 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 1-5 years, that there should be no concern regarding the withdrawal period.

Note: an amendment to the study protocol was submitted to CVM by the AADAP Office on April 29, 2009 requesting “repetitive treatments” be allowed. CVM response letter I-010969-X-0034-CE dated Aug. 7, 2009 was received by the AADAP Office and we are in the process of responding back to its comments.

Fish Species Treated and Fish Diseases Involved in CY08 Trials

1. Species and size of fish treated

Seven fish species, including one species of salmonid and six non-salmonids fish species, were treated during CY08. Treated fish ranged in length from 0.75 - 10.0 in. and the average length of all treated fish was 3.30 in. Species treated included:

Salmonid:

1. kokanee salmon kokanee salmon *Oncorhynchus nerka*

Non-salmonids:

1. channel catfish *Ictalurus punctatus*
2. fathead minnow *Pimephales promelas*
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 90% of the trials; while BGD was treated for in 10% 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 90% of the trials submitted in CY08.

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 AADAP Office for review, data analysis

and report writing, entering data into a database, and archiving in permanent files.

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 10 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.

Discussion of Study Results

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 4 provide a summary of all trials conducted during CY08 under INAD #10-969).

A. Efficacy of DQT When Used to Treat Columnaris

A total of 18 trials were conducted in which channel catfish, fathead minnows, largemouth bass, muskellunge, and walleye were diagnosed with columnaris and treated with DQT at doses that ranged from 4 to 28 mg/L DQT for durations that ranged from 1 to 4 h. Fish were treated over a period that extended from 1 to 58 days (see Tables 1 - 2). Diquat treatments appeared effective in 16 trials while two trials were characterized as inconclusive.

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

Two trials were conducted in which kokanee salmon and northern pike were diagnosed with BGD and were treated with DQT at doses that ranged from 10 to 20 mg/L DQT for a 1 h duration. Fish were treated over a period that extended from 6 to 16 days (see Table 1). Both of these trials appeared to be effective.

3. Observed Toxicity

No toxicity or adverse effects relating to DQT treatments were reported in any of the trials.

Current Study Protocol for Diquat (Reward®) INAD #10-969

Please see the attached current study protocol for Diquat (Reward®) INAD #10-969. Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see "Table 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the Diquat (Reward®) INAD #10-969 during CY08.

The following facility had diquat on-hand during CY08 but never used the drug:

1. Richloam SFH

Correspondence sent to Diquat (Reward®) INAD #10-969 Participants

Please see the attached correspondence that was sent to all diquat participants after the AADAP Office received their sign-up form for calendar year 2008.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY08 was 3,783,506. The total number of treated fish to count against the current treatment use authorization dated October 31, 2007 is 3,876,980.

Summary of Study Results

Diquat (Reward®) was used at doses ranging from 4 to 28 mg/L in 20 trials to control mortality in a variety of fish species caused by either columnaris or BGD. Fish were treated 1 - 58 times on consecutive or alternate days for durations that ranged from 1 to 4 h. Treatments were administered to seven different fish species, and treatment trials

involved approximately 3.8 million fish. Mean length of fish treated during CY08 was 3.3 in (range, 0.75 - 10.0 in), and mean water temperature of all trials was 69.8 ^{o}F (range, 51.0 - 79.3 ^{o}F). Results from approximately 90% of trials indicated that DQT treatments appeared effective in controlling mortality while 10% of the trials were characterized as inconclusive. Investigators reported no evidence of toxicity or adverse effects related to DQT treatment in any of the trials. 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 continue to be directed towards the generation of high quality data.

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 CY08 Diquat (Reward®) Field Efficacy Trial Results - Effective Treatments

Hatchery	Number of efficacious trials	Fish Species	Fish Size (in.)	Number of Fish	Disease	Dose (mg/L)	Duration (hrs)	Number of treatment days	Temp. (°F)
Rathbun SFH	2	CCF	6.9 - 8.1	17,988	Columnaris	4	4	1	65.5 - 75.0
	5	CCF	1.8 - 2.1	743,870	Columnaris	12 - 18	2 - 4	1 - 37	68.7 - 79.3
Ford SFH	1	KOE	1.80	985,000	BGD	10 - 20	1	6 - 12	51.0
Jake Wolf Memorial SFH	2	LMB	3.8 - 4.3	33,324	Columnaris	28	1	3	75.0 - 78.0
Jake Wolf Memorial SFH	1	MUE	3.50	64,500	Columnaris	28	1	19	68.0
Rathbun SFH	1	MUE	10.00	3,911	Columnaris	18	4	3	58.0
Spirit Lake SFH	1	MUE	0.75	114,493	Columnaris	10 & 18	1	34 - 58	69.6
Jake Wolf Memorial SFH	1	NOP	4.00	18,928	Columnaris	27.1	1	5	68.0
Spirit Lake SFH	1	NOP	0.75	750,000	BGD	10	1	16	61.6
Rathbun SFH	2	WAE	2.7 - 3.0	593,123	Columnaris	10 - 28	1	2 - 24	72.0 - 73.2
Spirit Lake SFH	1	WAE	3.80	42,043	Columnaris & BGD	10 & 18	1	16 - 20	70.3

Table 2. Summary of CY08 Diquat (Reward®) Field Efficacy Trial Results - Inconclusive Treatments

Hatchery	Number of inconclusive trials	Fish Species	Fish Size (in.)	Number of Fish	Disease	Dose (mg/L)	Duration (hrs)	Number of treatment days	Temp. (°F)
Rathbun SFH	1	FHM	2.00	300,000	Columnaris	12	4	4	58.0
Rathbun SFH	1	WAE	1.00	116,326	Columnaris	12 - 20	1 - 2	2 - 19	74.6

Table 3. Summary of Number of Treated Fish, Number of Treatment Trials, Treatment Regimens Used, and Fish Species Treated during CY08 Diquat (Reward®) Field Efficacy Trials

Total Number of Fish Treated:	**3,783,506**
Number of fish treated in effective trials	3,367,180
Number of fish treated in inconclusive trials	416,326
Total Number of Trials:	**20**
Number of trials in which treatments were effective	18
Number of trials in which treatment results were inconclusive	2

Treatment Regimes and Frequency Used:

4 mg/L for 4 hr; 1day	2 trials
10 mg/L for 1 hr; 16 days	1 trial
10;18 mg/L for 1 hr; 16 - 58 days	2 trials
10;15;20 mg/L for 1 hr; 6 - 12 days	1 trial
10;12;15;18;22;28 mg/L for 1 hr; 2 - 14 days	1 trial
12 mg/L for 2 - 4 hr; 1 - 4 days	2 trials
12;18 mg/L for 4 hr; 2 - 7 days	1 trial
12;15;18;20 mg/L for 1 hr; 2 - 19 days	1 trial
12;18;20;28 mg/L for 1 hr; 3 - 24 days	1 trial
15 mg/L for 2 hr; 1 day	1 trial
15;18 mg/L for 4 hr; 5 - 37 days	2 trials
18 mg/L for 4 hr; 3 days	1 trial
27.1 - 28 mg/L for 1 hr; 3 - 19 days	4 trials

Treatment Water Temperature (^{o}F):

Temperature Range	51.0 - 79.3
Mean Temperature	69.8

Size of Treated Fish (in.):

Fish Size Range	0.75 - 10.0
Mean Fish Size	3.3

Species Treated:

Salmonid:
kokanee salmon kokanee salmon *Oncorhynchus nerka*

Non-salmonids:
channel catfish *Ictalurus punctatus*
fathead minnow *Pimephales promelas*
largemouth bass *Micropterus salmoides*
muskellunge *Esox masquinongy*
northern pike *E. lucius*
walleye *Stizostedion vitreum*