

Diquat (Reward⁷) Clinical Field Trials - INAD #10-969

Year 2012 - 2014 Annual Summary Report on the Use of Diquat (Reward⁷) in Clinical Field Efficacy Trials

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Summary

Diquat (Reward⁷) 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 years 2012 - 14 (CY12-14) the efficacy of diquat (DQT) was evaluated in 201 disease trials involving approximately 14.7 million fish to control mortality in a variety of fish species caused by external columnaris or external coldwater disease. Trials were conducted at three state fish hatcheries and one private facility. 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 78% of the trials, ineffective in 2% of the trials, and characterized as inconclusive in 20% of the trials.

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.

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 CY12-14.

Purpose of Report

The purpose of this report is to summarize the results of DQT field efficacy trials conducted under INAD #10-969 during CY12-14. 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 three state fish hatcheries and one private facility used DQT to control mortality in fish caused by external columnaris or external coldwater disease. Mean water temperature during all treatments was 73.8 EF, and water temperature for individual trials during treatments at the various testing facilities ranged from 47.0 – 85.8 EF.

2. Chemical material

REWARD⁷ (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 CY12-14 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 (Reward⁷) 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 (Reward⁷) was used by investigators at one or both of the following dosage regimens:

1. 4 - 18 mg/L for 0.5 hr - 4 hr
2. 19 - 28 mg/L for 0.5 - 1 hr

Note: the investigators noted that the treatment duration (hrs) was followed for all treatment dosages; even when different doses were used during a treatment period. For example, when treatment doses were between 19 mg/L and 28 mg/L the treatment duration was only for 0.5 - 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 dosage of 2 - 18 mg/L or (2) 1 - 3 times on consecutive days at a dosage of 19 - 28 mg/L. Note: an amendment to the study protocol

was accepted by CVM on April 15, 2010 allowing Arepetitive treatments@ at certain facilities.

Fish Species Treated and Fish Diseases Involved in CY12-14 Trials

1. Species and size of fish treated

Seven non-salmonid fish species were treated during CY12-14. Treated fish ranged in length from 0.5 - 22 in. and the average length of all treated fish was 4.4 in. Species treated included:

Non-salmonids:

channel catfish (*Ictalurus punctatus*)

fathead minnow (*Pimephales promelas*)

largemouth bass (*Micropterus salmoides*)

muskellunge (*Esox masquinongy*)

northern pike (*E. Lucius*)

walleye (*Sander vitreus*)

yellow perch (*Perca flavescens*)

2. Diseases treated

The fish disease treated most frequently was characterized as external columnaris, which was treated for in 99% of the trials; while external coldwater disease was treated for in 1% of the 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. No pathology reports were submitted in CY12-14.

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 through the online INAD database. Such reports were routed through the study monitor for review, and then sent to the AADAP Office for review, data analysis and report writing, 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 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 DQT treatments were ineffective; Table 3 provides a summary of all trials in which DQT treatments were inconclusive; and Table 4 provides a summary of all treatment trials, including number of trials, number of fish treated, and treatment regimens used during CY12-14 under INAD #10-969).

A. Efficacy at 4 - 28 mg/L for 0.5 to 4hrs duration for 1 – 75 days in non-salmonid fish

A total of 201 trials were conducted in which channel catfish, fathead minnows, largemouth bass, muskellunge, northern pike, walleye, and yellow perch were diagnosed with external columnaris or external coldwater disease and treated with DQT at doses that ranged from 4 to 28 mg/L DQT for durations that ranged from 0.5 to 4 h. Fish were treated over a period that extended from 1 to 75 days (see Tables 1 - 3). Trials appeared to be effective in 157 trials, ineffective in 4 trials, and characterized as inconclusive in 40 trials.

2. Observed Toxicity

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

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for Diquat (Reward⁷) INAD #10-969

No changes have occurred to the current study protocol for Diquat (Reward⁷) INAD #10-969.

Facility Sign-up List

Please see ATable 5. Facilities and Names of Investigators@ for facilities that signed-up to participate in the Diquat (Reward⁷) INAD #10-969 during CY12-14. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

Correspondence sent to Diquat (Reward⁷) 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 CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 14,746,828. However, it should be noted that the fish treated from the facilities were re-treated multiple times. The actual number of fish treated once to count against the authorization is unknown, but is lower than the number reported. The actual number of fish treated the first time is now being tracked by the facilities so future authorization numbers should be accurate. The total number of treated fish to count

against the treatment use authorization dated August 5, 2009 is 10,000,000. It was determined that the total number of treated fish to count against the treatment use authorization dated June 11, 2014 is 4,569,966 (this is factoring in as closely as possible the number of re-treatments of fish so the actual number is only fish treated the first time).

Summary of Study Results

Diquat (Reward⁷) was used at doses ranging from 4 to 28 mg/L in 201 trials to control mortality in a variety of fish species caused by either columnaris or external coldwater disease. Fish were treated 1 - 75 times on consecutive or alternate days for durations that ranged from 0.5 to 4 h. Treatments were administered to seven different fish species, and treatment trials involved approximately 14.7 million fish. Mean length of fish treated during CY12-14 was 4.4 in, and mean water temperature of all trials was 73.8 °F. Results from 78% of the trials indicated that DQT treatments appeared effective in controlling mortality, while 2% were ineffective and 20% 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.

Table 1. Summary of CY12-14 Diquat (Reward⁷) Treatment Results - Efficacious Trials

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	16	Channel Catfish	1.4 – 8.0	978,462	Columnaris - external	4 - 15	3 - 4	1 - 7	53.6 – 81.3
Rathbun SFH	1	Fathead Minnow	2.0	100,000	Columnaris - external	12	4	4	47
Rathbun SFH	1	Largemouth Bass	4.9	4,322	Columnaris - external	12	3	3	53.6
Rathbun SFH	5	Muskellunge	6.9 – 12.5	23,352	Columnaris - external	12 - 18	3 - 4	3 - 4	47.0 – 64.0
Spirit Lake SFH	3	Muskellunge	6.0 – 11.0	269,571	Columnaris - external	10 - 18	1	12 - 75	65.0 – 71.5
Spirit Lake SFH	1	Muskellunge	10.0	106,155	External CWD - Bacterial Coldwater Disease	10 - 18	1	52	75.0
Jake Wolf SFH	1	Northern Pike	5.0	16,388	Columnaris - external	28	1	3	68.0
Spirit Lake SFH	2	Northern Pike	3.0 – 5.0	817,987	Columnaris - external	10 - 18	1	32 - 37	60.1 – 61.3
Spirit Lake SFH	1	Northern Pike	4.3	694,690	External CWD - Bacterial Coldwater Disease	10 - 18	1	41	60.9
Rathbun SFH	113	Walleye	0.8 – 22.0	3,345,480	Columnaris - external	12 - 28	1 - 4	1 - 40	53.6 – 85.5
Spirit Lake SFH	2	Walleye	5.0 – 6.0	102,014	Columnaris - external	10 - 18	1	17 - 25	70.7 – 74.5
Spirit Lake SFH	1	Walleye	4.0	50,000	External CWD - Bacterial Coldwater Disease	10 - 18	1	18	68.4
Bell Aquaculture	10	Yellow Perch	3.5 – 6.7	3,761,855	Columnaris - external	5.3 - 19	0.5 – 1.5	3 - 4	64.4 – 73.4

Table 2. Summary of CY12-14 Diquat (Reward⁷) Treatment Results – Ineffective Trials

Hatchery	Number of ineffective trials	Fish Species	Fish Size (in.)	Number of Fish	Disease	Dose (mg/L)	Duration (hrs)	Number of treatment days	Temp. (°F)
Rathbun SFH	2	Walleye	2.9 – 6.9	30,450	Columnaris - external	18 - 28	1 - 3	3 - 5	74.3 – 85.8
Bell Aquaculture	2	Yellow Perch	0.5 – 6.7	610,000	Columnaris - external	5.0 – 5.3	0.5 - 1	4	66.2

Table 3. Summary of CY 2012 - 2014 Diquat (Reward⁷) Treatment Results - Inconclusive Trials

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	Channel Catfish	4	40,000	Columnaris - external	15	3	41	75.0
Rathbun SFH	1	Fathead Minnow	2.4	2,826	Columnaris - external	15	4	3	70.7
Rathbun SFH	33	Walleye	1.9 – 20.9	573,299	Columnaris - external	12 - 28	1 - 4	1 - 11	48.9 – 79.0
Bell Aquaculture	5	Yellow Perch	5.5 – 6.7	3,219,977	Columnaris - external	5.3 – 11.3	0.5 - 1	2 - 4	62.6 – 68.0

Table 4. Summary of Number of Treated Fish, Number of Treatment Trials, Treatment Regimens Used, and Fish Species Treated during CY12-14 Diquat (Reward⁷) Field Efficacy Trials

Total Number of Fish Treated:	14,746,828
Number of fish treated in effective trials	10,270,276
Number of fish treated in ineffective trials	640,450
Number of fish treated in inconclusive trials	3,836,102

Total Number of Trials:	201
Number of trials in which treatments were effective	157
Number of trials in which treatments were ineffective	4
Number of trials in which treatments were inconclusive	40

Treatment Regimes and Frequency Used:	
4 - 28 mg/L for 0.5 - 4 hr; 1 - 75 days	201 trials
4 - 18 mg/L for 0.5 - 4 hrs	
19 - 28 mg/L for 0.5 - 1 hr	

Treatment Water Temperature (°F):	
Temperature Range	47.0 - 85.8
Mean Temperature	73.8

Size of Treated Fish (in.):	
Fish Size Range	0.5 - 22.0
Mean Fish Size	4.4

Species Treated:

Non-salmonids:

channel catfish (*Ictalurus punctatus*)
 fathead minnow (*Pimephales promelas*)
 largemouth bass (*Micropterus salmoides*)
 muskellunge (*Esox masquinongy*)
 northern pike (*E. Lucius*)
 walleye (*Sander vitreus*)
 yellow perch (*Perca flavescens*)