

**Hydrogen Peroxide (35% PEROX-AID®) Clinical Field Trials -
INAD 11-669**

**Year 2009 Annual Summary Report on the Use of Hydrogen Peroxide
(35% PEROX-AID®) in Clinical Field Efficacy Trials**

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Summary

Hydrogen Peroxide (35% PEROX-AID®) has been used effectively in the U. S. under compassionate INAD Exemption #11-669 to control mortality in a variety of fish caused by common fish bacterial pathogens or ectoparasites. In calendar year 2009 (CY09), the efficacy of Hydrogen Peroxide (35% PEROX-AID®) (H₂O₂) was evaluated in 36 ectoparasite trials involving approximately 1.4 million fish to control mortality in Kona kampachi caused by ectoparasites. Trials were conducted at one private fish hatchery. The compassionate study protocol under which treatments were administered allowed the investigator to use H₂O₂ on either three consecutive or alternate days for 0.5 - 1h at dosages ranging from 50 - 200 mg/L; or one day a week for 0.75 h at 400 mg/L. Overall, results of trials conducted in CY09 indicated that treatments appeared efficacious in all trials

Introduction

The current labels for H₂O₂ use in aquaculture limits use to: 1) Freshwater-reared finfish eggs to control mortality due to saprolegniasis; 2) Freshwater-reared salmonids to control mortality due to bacterial gill disease; and 3) Freshwater-reared coolwater finfish and channel catfish to control mortality due to external columnaris disease. These label restrictions limit the overall utility of approved H₂O₂ use in aquaculture.

External parasites (ectoparasites) form one of the largest groups of pathogenic organisms of cultured aquatic species (Post 1987). Affected species include finfish (freshwater and marine) and invertebrates. Environmental conditions such as temperature change, poor water quality, and high organic loading due to intensive fertilization and feeding levels increase the incidence and spread of many external parasites. Stress (i.e., seining, handling, sorting, grading, vaccinating, anesthesia, crowding, and transport) is also a major contributor to most parasitic outbreaks in fish (Lasee 1995). Additionally, tissue damage induced by external parasites increases susceptibility to secondary bacterial and/or fungal infections (Lasee, 1995).

The organisms responsible for major parasitic infections on fish are, for the most part, protozoan and metazoan. The parasites affecting the external surface of fish typically include those of the genera *Ambiphrya*, *Chilodonella*, *Cleidodiscus*, *Dactylogyrus*, *Epistylis*, *Gyrodactylus*, *Ichthyobodo*, *Ichthyophthirius*, *Trichodina*, and *Trichophrya*. These parasites are highly opportunistic and have tremendous reproductive capabilities. Under normal conditions (e.g., in wildstock populations) these

organisms cause little pathology. However, under intensive culture where fish densities are typically high, many of these organisms can cause serious disease problems.

If parasitic infections are left untreated, they can cause substantial economic losses to commercial aquaculture, and severely impact the restoration, recovery, and preservation of depleted stocks of fish cultured by Federal and State agencies. The extent of losses of fish from parasites depends upon the severity of the primary cause of infection. Morbidity can vary from less than 10% to total loss of the population (Post 1987). Historically, immersion treatments (static and flush) using a variety of compounds have been used to control mortality caused by parasite infestations. A number of these compounds have been found, both experimentally and under production settings, to be relatively effective.

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.

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°C, epizootics often occur in cultured fishes at 10 - 17°C.

Flavobacterium 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).

Purpose of Report

The purpose of this report is to summarize the results of CY09 supplemental H₂O₂ field efficacy data. We anticipate that CY09 data will be used to enhance the existing H₂O₂ 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 H₂O₂ in aquaculture.

Facilities, Materials, Treatment Procedures

1. Facilities

A total of 36 field efficacy trials were conducted at one private fish hatchery. Treatments were used to control mortality caused by ectoparasites in one fish species. Water temperature during treatments at the various testing facilities ranged from 78.0 - 80.0 °F, with a mean treatment temperature of 79.2°F.

2. Chemical material

H₂O₂ (CAS No. 7722-84-1) is a clear colorless liquid that contains 35% hydrogen peroxide. All facilities used designated lots of H₂O₂ provided by Western Chemical, Inc, Ferndale, WA.

3. Treatment Methods

H₂O₂ treatments were administered using either a flow-through or standing bath treatment method. Both procedures called for accurately weighed amounts of liquid chemical to be pre-mixed in an appropriate amount of non-chlorinated water. 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 0.5 - 1 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 H₂O₂ concentration throughout the tank. Thorough mixing was essential to ensure there were no H₂O₂ "hot spots." After the treatment, water flow was turned on again to flush the chemical out of the rearing unit.

4. Drug dosages

During CY09, one H₂O₂ dosage treatment regimen was used. Listed below is the dose and the number of trials conducted:

1. 400 mg/L; 0.75 hr 36 trials

5. Number of treatments per disease outbreak

According to the Study Protocol, Investigators were allowed to administer H₂O₂ on (1) 1 - 3 consecutive/alternating days when used at a dosage of 50 - 200 mg/L; or (2) 1 time/wk at a dosage of 400 mg/L when used on marine fish species.

Fish Species Treated and Fish Diseases Involved in CY09 Trials

1. Species and size of fish treated

One marine non-salmonid fish species was treated during CY09. Treated fish ranged in length from 3.5 - 24.0 in. and the average length of all treated fish was 15.4 in. Kona kampachi (*Seriola rivoliana*) was the only species treated during CY09.

2. Ectoparasite treated

Test fish were treated with H₂O₂ to control mortality caused by ectoparasites of the genera *Neobenedenia*.

Data Collected

1. Pathologist's report

Fish health pathology reports provide essential information with respect to parasite confirmation and general fish health. No pathology reports were submitted with the CY09 trials.

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 28 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 fish shortly after final treatment.

Discussion of Study Results

- 1. General observations on the efficacy of H₂O₂ for the control of ectoparasites in non-salmonid marine fish** (Note: Table 1 provides a summary of all trials in which treatment appeared efficacious; Table 2 provides summary data for all trials; and Table 3 provides a brief description of all trials conducted during CY09 under INAD #11-669).

A. Efficacy at 400 mg/L H₂O₂

Kona Kampachi were treated with 400 mg/L H₂O₂ for a 0.75 h duration for 1 - 3 days in 36 trials (Table 1). Investigators used H₂O₂ to control mortality caused by ectoparasites of the genera *Neobenedenia*. H₂O₂ treatments appeared effective in all trials.

2. Observed Toxicity

No toxicity or adverse effects relating to H₂O₂ treatment were reported in any of the trials.

3. Observed Withdrawal Period

No withdrawal time is needed for fish treated with H₂O₂ under the current Food-Use Authorization dated June 23, 2010.

Current Study Protocol for Hydrogen Peroxide (35% PEROX-AID®) INAD #11-669

Please see the attached current study protocol for Hydrogen Peroxide (35% PEROX-AID®) INAD #11-669. Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 4. Facilities and Names of Investigators” for facilities that signed-up to participate in the Hydrogen Peroxide (35% PEROX-AID®) during CY09. Facilities not listed in Appendix III-a of the current Hydrogen Peroxide (35% PEROX-AID®) INAD #11-669 during CY09 study protocol have been highlighted. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

Correspondence sent to Hydrogen Peroxide (35% PEROX-AID®) INAD #11-669

Participants

Please see the attached correspondence that was sent to all H₂O₂ participants after the AADAP Office received their sign-up form for CY09.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY09 was 1,383,541. The total number of treated fish to count against the current treatment use authorization dated December 19, 2007 is 2,207,401.

Summary of Study Results

H₂O₂ was used at a dosage of 400 mg/L in 36 treatment trials in which fish were treated one to three times to control mortality. Kona kampachi was the only fish species treated and trials involved approximately 1.4 million fish. Treated fish ranged in size from 3.5 - 24.0 in. Water temperature during treatment ranged from 78.0 - 80.0°F, with a mean treatment temperature of 79.2°F. Overall, results showed that treatment appeared effective in all of the trials. There was no evidence of toxicity or adverse effects related to H₂O₂ treatment reported in any of the trials. Data from the CY09 trials indicate that the H₂O₂ treatment regimen recommended in INAD Protocol #11-669 is safe and effective to control mortality in Kona kampachi caused by ectoparasites. As a result of the lack of quality criteria, such as dose verification, use of controls, replicates, and randomization, it is understood that these data will be considered as ancillary data, and that pivotal efficacy studies are needed to definitively demonstrate H₂O₂ efficacy for the treatment of ectoparasites. However, the ancillary data described above should provide useful, corroborative data to help support a label claim for the use of H₂O₂ to control mortality associated with ectoparasites in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #11-669 will also be ancillary data, efforts will be directed towards the continued generation of high quality data.

References

Lasee, B. A., editor. 1995. *Introduction to Fish Health Management*, 2nd edition. U.S. Fish and Wildlife Publication. Washington, D.C. 139 pp.

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

Table 1. Summary of Year 2009 H₂O₂ Efficacy Results - Efficacious Studies

Hatchery	Number of efficacious trials	Fish Species	Fish Size (in.)	Number of Fish	Ectoparasite	Dose (mg/L)	Duration (hrs)	Number of treatment days	Withdrawal Period (days)	Temp. (°F)
Kona Blue Water Farms	36	KON	3.5 - 24.0	1,383,541	Neobenedenia	400	0.75	1 - 3	0 - 287	78.0 - 80.0

Table 2. Summary Data Regarding Year 2009 H₂O₂ Efficacy Studies

Total Number of Fish Treated:	1,383,541
Number of fish treated in efficacious trials	1,383,541
Total Number of Studies:	36
Efficacious trials	36
Treatment Regimens and Frequency Used:	
400 mg/L; 0.75 hr; 1 - 3 days	36 trials
Treatment Water Temperature (°F):	
Temperature Range	78.0 - 80.0
Mean Temperature	79.2
Size of Treated Fish (in.):	
Size Range	3.5 - 24.0
Mean Length	15.4
Species Treated:	
<u>Marine non-salmonids:</u>	
kona kampachi <i>Seriola rivoliana</i>	
