

**Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) Clinical Field Trials -**  
**INAD 11-669**

**Year 2012 - 2014 Annual Summary Report on the Use of Hydrogen Peroxide  
(35% PEROX-AID<sup>7</sup>) in Clinical Field Efficacy Trials**

Prepared by:

Bonnie Johnson, Biologist  
U. S. Fish and Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Bozeman, Montana

**Summary**

Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) has been used effectively in the U. S. under compassionate INAD Exemption #11-669 to control mortality in a variety of fish caused by ectoparasites. In calendar years 2012 - 2014 (CY12-14), the efficacy of Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) (H<sub>2</sub>O<sub>2</sub>) was evaluated in 121 ectoparasite trials involving approximately 8.2 million fish to control mortality in Hawaiian kampachi, cutthroat trout, and rainbow trout caused by ectoparasites. Trials were conducted at one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), one state fish hatchery, and one private fish hatchery. The compassionate study protocol under which treatments were administered allowed the investigator to use H<sub>2</sub>O<sub>2</sub> on either three consecutive or alternate days for 0.5 - 1hr at dosages ranging from 50 - 200 mg/L; or one day a week for 0.75 hr at 400 mg/L. Overall, results of trials conducted in CY12-14 indicated that treatments appeared efficacious in 91% of the trials and ineffective in 9% of the trials.

## Introduction

The current labels for H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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.

### **Purpose of Report**

The purpose of this report is to summarize the results of CY12-14 supplemental H<sub>2</sub>O<sub>2</sub> field efficacy data. We anticipate that CY12-14 data will be used to enhance the existing H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> in aquaculture.

### **Facilities, Materials, Treatment Procedures**

#### **1. Facilities**

A total of 121 field efficacy trials were conducted at one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), one state fish hatchery, and one private fish hatchery. Treatments were used to control mortality caused by ectoparasites in three fish species. Water temperature during treatments at the various testing facilities ranged from 44.0 – 80.6 EF, with a mean treatment temperature of 73.6EF.

## **2. Chemical material**

H<sub>2</sub>O<sub>2</sub> (CAS No. 7722-84-1) is a clear colorless liquid that contains 35% hydrogen peroxide. All facilities used designated lots of H<sub>2</sub>O<sub>2</sub> provided by Western Chemical, Inc, Ferndale, WA.

## **3. Treatment Methods**

H<sub>2</sub>O<sub>2</sub> treatments were administered in both freshwater and in saltwater sea cages.

H<sub>2</sub>O<sub>2</sub> treatments that were administered in freshwater used 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 H<sub>2</sub>O<sub>2</sub> concentration throughout the tank. Thorough mixing was essential to ensure there were no H<sub>2</sub>O<sub>2</sub> "hot spots." After the treatment, water flow was turned on again to flush the chemical out of the rearing unit.

Immersion treatments that were treated in saltwater entailed raising the sea cage to the equatorial rim and using a 12-pieced pie-shaped tarp clipped together at each abutment to

contain therapeutant, water, and fish. Because the tarp is not one continuous piece and is porous, there is considerable dilution of therapeutant concentration that occurs throughout the treatment period. This situation is further exacerbated by direct exposure of the sea cages to ocean currents.

#### **4. Drug dosages**

During CY12-14, four H<sub>2</sub>O<sub>2</sub> dosage treatment regimens were used. Listed below are the dose and the number of trials conducted:

1. 50 mg/L; 0.50 – 1.0 hr; 4 trials; freshwater
2. 70 mg/L; 0.50 hr; 4 trials; freshwater
3. 100 mg/L; 0.50 hr; 3 trials; freshwater
4. 400 mg/L; 0.75 hr; 110 trials; saltwater sea cages

#### **5. Number of treatments per disease outbreak**

According to the Study Protocol, Investigators were allowed to administer H<sub>2</sub>O<sub>2</sub> 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 in sea cages.

### **Fish Species Treated and Fish Ectoparasites Involved in CY12-14 Trials**

#### **1. Species and size of fish treated**

Three fish species, one marine non-salmonids and two salmonids, were treated during CY12-14. Treated fish ranged in weight from 20 – 3,770 g; mean weight was 1,103.6 g.

Species treated included:

**Marine non-salmonids**

Hawaiian kampachi (*Seriola rivoliana*)

**Salmonid**

rainbow trout (*Oncorhynchus mykiss*)

cutthroat trout (*O. clarki*)

**2. Ectoparasite treated**

Test fish were treated with H<sub>2</sub>O<sub>2</sub> to control mortality caused by ectoparasites of the genera *Neobenedenia* or *Gyrodactylus*.

**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 CY12-14 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 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 28 d post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis.

## **Discussion of Study Results**

- 1. General observations on the efficacy of H<sub>2</sub>O<sub>2</sub> for the control of ectoparasites in treated fish** (Note: Table 1 provides a summary of all trials in which treatment appeared efficacious; Table 2 provides a summary of all trials in which treatment appeared ineffective; and Table 3 provides summary data for all trials conducted during CY12-14 under INAD #11-669).

### **A. Efficacy at 50 - 100 mg/L H<sub>2</sub>O<sub>2</sub>**

Rainbow trout and cutthroat trout were treated with 50 - 100 mg/L H<sub>2</sub>O<sub>2</sub> for a 0.5 – 1 hr duration for 3 consecutive or alternating days in 11 trials (Table 1). Investigators used H<sub>2</sub>O<sub>2</sub> to control mortality caused by ectoparasites of the genera *Gyrodactylus*. H<sub>2</sub>O<sub>2</sub> treatments appeared effective in all trials.

### **B. Efficacy at 400 mg/L H<sub>2</sub>O<sub>2</sub>**

Hawaiian Kampachi were treated with 400 mg/L H<sub>2</sub>O<sub>2</sub> for a 0.75 hr duration for 1 day in 110 trials (Tables 1 - 2). Investigators used H<sub>2</sub>O<sub>2</sub> to control mortality caused by ectoparasites of the genera *Neobenedenia*. H<sub>2</sub>O<sub>2</sub> treatments appeared effective in 99 trials and ineffective in 11 trials.

## **2. Observed Toxicity**

No toxicity or adverse effects relating to H<sub>2</sub>O<sub>2</sub> treatment were reported in any of the trials.

## **3. Observed Withdrawal Period**

No withdrawal time is needed for fish treated with H<sub>2</sub>O<sub>2</sub> under the Food-Use Authorization dated June 23, 2010.

### **Current Study Protocol for Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) INAD #11-669**

No changes have occurred to the current study protocol for Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) INAD #11-669.

### **Facility Sign-up List**

Please see ATable 4. Facilities and Names of Investigators@ for facilities that signed-up to participate in the Hydrogen Peroxide (35% PEROX-AID<sup>7</sup>) during CY12-14. 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<sup>7</sup>) INAD #11-669**

### **Participants**

Please see the attached correspondence that was sent to all H<sub>2</sub>O<sub>2</sub> 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 8,239,432. The total number of treated fish to count against the current treatment use authorization dated December 19, 2007 is 15,546,433.

### **Summary of Study Results**

H<sub>2</sub>O<sub>2</sub> was used at a dosage of 50 - 400 mg/L in 121 treatment trials in which fish were treated one to three times to control mortality. Hawaiian kampachi, cutthroat trout, and rainbow trout were the only fish species treated and trials involved approximately 8.2 million fish. Treated fish ranged in weight from 20 – 3,770 g. Water temperature during treatment ranged from 44.0 – 80.6°F, with a mean treatment temperature of 73.6°F. Overall, results showed that treatment appeared effective in 91% of the trials and was ineffective in 9% of the trials. There was no evidence of toxicity or adverse effects related to H<sub>2</sub>O<sub>2</sub> treatment reported in any of the trials. Data from the CY12-14 trials indicate that the H<sub>2</sub>O<sub>2</sub> treatment regimen recommended in INAD Protocol #11-669 is safe and effective to control mortality in fish 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 H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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 2012 - 2014 H<sub>2</sub>O<sub>2</sub> Efficacy Results - Efficacious Studies**

Hatchery	Number of efficacious trials	Fish Species	Fish Weight (g)	Number of Fish	Ectoparasite	Dose (mg/L)	Duration (hrs)	Number of treatment days	Temp. (°F)
Leadville NFH	1	CUT	75.6	39,000	Gyrodactylus	50	1.0	3	44.0
Boulder Rearing Station	3	RBT	473 – 1,135	5,800	Gyrodactylus	50	0.5	3	52.0
Boulder Rearing Station	4	RBT	30.2 – 1,565	43,600	Gyrodactylus	70	0.5	3	52.0
Boulder Rearing Station	3	RBT	252 – 2,268	9,026	Gyrodactylus	100	0.5	3	52.0
Keahole Point Fish LLC	99	KON	20 – 3,770	6,979,906	Neobenedenia	400	0.75	1	71.6 – 80.6

**Table 2. Summary of Year 2012 - 2014 H<sub>2</sub>O<sub>2</sub> Efficacy Results - Ineffective Studies**

Hatchery	Number of ineffective trials	Fish Species	Fish Weight (g)	Number of Fish	Ectoparasite	Dose (mg/L)	Duration (hrs)	Number of treatment days	Temp. (°F)
Keahole Point Fish LLC	11	KON	20 – 1,456	1,162,100	Neobenedenia	400	0.75	1	73.6 – 80.6

**Table 3. Summary Data Regarding Year 2012 - 2014 H<sub>2</sub>O<sub>2</sub> Efficacy Studies**

<b>Total Number of Fish Treated:</b>	<b>8,239,432</b>
Number of fish treated in efficacious trials	7,077,332
Number of fish treated in ineffective trials	1,162,100
<b>Total Number of Studies:</b>	<b>121</b>
Efficacious trials	110
Ineffective trials	11
<b>Treatment Regimens and Frequency Used:</b>	
50 mg/L; 0.50 – 1.0 hr; 3 days	4 trials
70 mg/L; 0.5 hr; 3 days	4 trials
100 mg/L; 0.50 hr; 3 days	3 trials
400 mg/L; 0.75 hr; 1 day	110 trials
<b>Treatment Water Temperature (°F):</b>	
Temperature Range	44.0 – 80.6
Mean Temperature	73.6
<b>Weight of Treated Fish (g):</b>	
Weight Range	20 – 3,770
Mean Weight	1,103.6
<b>Species Treated:</b>	
<u><b>Marine non-salmonids:</b></u>	
Hawaiian kampachi <i>Seriola rivoliana</i>	
<u><b>Salmonid</b></u>	
rainbow trout ( <i>Oncorhynchus mykiss</i> )	
cutthroat trout ( <i>O. clarki</i> )	