

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION
FOR 35% PEROX-AID[®] (HYDROGEN PEROXIDE) FOR THE
CONTROL OF ECTOPARASITES (INAD #11-669)**

Sponsor:

U.S. Fish and Wildlife Service, Division of the National Fish Hatchery System

Sponsor Signature

Date Approved

Manufacturer:

Eka Chemicals Inc.
1775 West Oak Commons Court
Marietta, Georgia 30062-2254

Facility for Coordination of 35% PEROX-AID[®] INAD:

Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road
Bozeman, MT 59715

Proposed Starting Date:

December 1, 2007

Proposed Ending Date:

November 30, 2012

Study Director:

Mr. Jim Bowker

Study Director Signature

Date

Clinical Field Trial Location:

Facility: _____

Type or Print Name

Investigator: _____

Type or Print Name

Investigator Signature

Date

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR HYDROGEN PEROXIDE (35% PEROX-AID®) UNDER INAD #11-669

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy and safety of 35% PEROX-AID® administered as an immersion bath to control mortality caused by ectoparasites of the genera *Ambiphrya*, *Chilodonella*, *Dactylogyrus*, *Epistylis*, *Gyrodactylus*, *Ichthyobodo*, *Ichthyophthirius*, *Trichodina*, *Trichophrya*, *Argulus*, *Salmincola*, *Lernaea*, and *Ergasilus* in freshwater fish species; and of the genera *Neobenedenia*, *Amyloodinium*, *Cryptocaryon*, and *Uronema* in marine fish species.

II. SPONSOR

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: dave_erdahl@fws.gov

Manufacturer:

Eka Chemical Inc.
1775 West Oak Commons Court
Marietta, Georgia 30062-2254
Contact: David Lovetro
Phone: 1-800-241-3900 or 770-321-4198
Email: Dave.Lovetro@eka.com

Study Director:

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U.S. Fish and Wildlife Service - AADAP
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Email: jim_bowker@fws.gov

Clinical Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

INAD Study Monitors: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: December 1, 2007

Proposed Completion Date: November 30, 2012

V. BACKGROUND/PURPOSE

A. Background Information on protozoan and metazoan ectoparasites in aquatic species:

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.

B. Background information on formalin as an ectoparasiticide:

In 1986, the U.S. Food and Drug Administration (FDA) approved a new animal drug application (NADA) for the use of formalin to control external parasites (*Ichthyophthirius*, *Chilodonella*, *Costia*, *Scyphidia*, *Epistylis*, *Trichodina*, *Cleidodiscus*, *Gyrodactylus*, and *Dactylogyrus*) on several fish species (salmonids, catfish, largemouth bass, and bluegill) and to control fungal infections on the eggs of salmon, trout and esocids. More recently, in 2002 the formalin label claim for use as a parasiticide was expanded to include ".....for use on all finfish".

While formalin has proven to be an effective parasiticide, it is not a cure-all, nor the drug-of-choice in all situations. While formalin is an effective parasiticide, its use is somewhat limited by species-specific effectiveness and toxicity issues. Furthermore, in certain jurisdictions formalin is not considered the most environmentally friendly compound, and formalin effluent issues can be problematic. In some cases fishery managers have reported an inability to meet State and/or local effluent requirements, and there is growing public concern regarding its safety in the workplace. It is unlikely that this concern over the discharge and handling of formalin will soon (if ever) reverse itself.

C. Background information on hydrogen peroxide as an ectoparasiticide:

Hydrogen peroxide is a relatively safe compound, which is used as an antimicrobial agent in cheese production, in the treatment of drinking water, as a bleaching agent in the textile industry, and as an antiseptic and treatment for external parasites on fish (Marking et al. 1994). Hydrogen peroxide is active against a wide variety of other organisms, including bacteria, yeasts, viruses, fungi, and fungal spores (Marking et al. 1994).

Hydrogen peroxide has been used to treat freshwater fish for ectoparasites since the 1930s. Hydrogen peroxide has been used as a topical bath treatment for ectoparasites of fish (Kabata, 1985), and has been applied as a bath treatment for sea lice in farmed Atlantic salmon in the Faroe Islands, Norway and Scotland (Thomassen, 1993), as well as in Canada (personal communication, D. Lovetro). Hydrogen peroxide treatment has been shown to substantially reduce or eliminate infestations of *Ambiphrya* or *Gyrodactylus* on rainbow trout (Rach et al. 2000). A study on evaluating long-term, low-dose hydrogen peroxide treatment at 25 mg/L indicated this methodology to be an effective treatment for ectoparasites on African cichlids and perhaps other similar species of fish (Montgomery-Brock et al. 2004). Hydrogen peroxide at 200 mg/L was effective in killing the adult parasite *S. chrysophrii* taken from the gills of gilthead sea bream (*Sparus aurata* L.) during *in vitro* treatments (Sitjà-Bobadilla et al, 2006).

In a study conducted by Rach et al. 1997, test tanks containing brown trout, lake trout, channel catfish, and bluegill exhibited no mortalities when exposed to up to 500 mg/L hydrogen peroxide for 15 min every other day for 4 consecutive treatments. Investigations have found no evidence of toxicity from hydrogen peroxide to glochidia of the plain pocketbook mussel *Lampsilis cardium* during encystment on largemouth bass *Micropterus salmoides* when hydrogen peroxide was applied at 100 mg/L for 60 min every other day for 3 treatments (Rach et al. 2006). Species sensitivity varies widely (Gaikowski et al. 1999; Rach et al. 1997) although tolerance of hydrogen peroxide can be increased by low level pre-exposure (Tort et al. 1998). Hydrogen peroxide has relatively little environmental impact as it breaks down into water and oxygen (Treasurer et al, 1997) and is relatively safe for users because no harmful fumes are released during application (Rach et al. 1997).

To date, much work has been done to support the development of a New Animal Drug Application (NADA) approval for hydrogen peroxide to control mortality caused by fungal, bacterial, and ectoparasitic diseases in a number of freshwater fish species. In January 2007 this work resulted in the approval of hydrogen peroxide (35% PEROX-AID[®]) for:

1. Control of mortality caused by saprolegniasis in freshwater-reared finfish eggs,
2. Control of mortality caused by bacterial gill disease in freshwater-reared salmonids, and
3. Control of mortality caused by external columnaris in freshwater-reared coolwater finfish and channel catfish.

The ultimate goal of the NADA sponsor is pursue labeling for 35% PEROX-AID[®] as an external microbiocide for all finfish and finfish eggs. Hence, this INAD is intended to assist in the gathering of data to extend the 35% PEROX-AID[®] label beyond the currently approved claims, and more specifically, to generate data supporting the labeling of 35% PEROX-AID[®] for use to control mortality caused by ectoparasites.

D. Purpose of INAD:

The primary purpose of this INAD for 35% PEROX-AID[®] administered as an aqueous flow-through or static immersion bath is to develop clinical field trial data that will be used to demonstrate the efficacy and safety of 35% PEROX-AID[®] treatment to control mortality caused by ectoparasites in a variety of freshwater and marine fish species under a variety of environmental conditions. These data will be used to support a new animal drug application (NADA) for 35% PEROX-AID[®].

The USFWS anticipates that it may take several years to complete all technical section data requirements for a NADA for 35% PEROX-AID[®] to control mortality caused by ectoparasites in freshwater and marine fish species. The USFWS is aware that opportunities for 35% PEROX-AID[®] therapy are unpredictable. There is no way of knowing in advance if, when, or where opportunities for pivotal studies will be encountered. The USFWS believes it is likely that data from 3-5 treatment seasons will be required in order

to adequately assess the efficacy of 35% PEROX-AID[®] treatment, and to generate sufficient data to support a NADA(s).

VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the effectiveness and safety of 35% PEROX-AID[®] administered as an aqueous flow-through or static immersion bath to control mortality caused by ectoparasites in a variety of freshwater and marine fish species.
2. Provide the opportunity for fishery biologists to legally use 35% PEROX-AID[®] to control mortality caused by ectoparasites in a variety of freshwater and marine fish species in order to maintain healthy stocks of fish during the period of time necessary for the collection of data that will be used to support a NADA(s) for 35% PEROX-AID[®] use in fish.

Under major objective #1, there are two specific Study Protocol objectives:

Objective A – Treatment of Ectoparasites in Freshwater Species

To determine the efficacy and safety of 35% PEROX-AID[®] treatment to control mortality caused by ectoparasites of the genera *Ambiphrya*, *Chilodonella*, *Dactylogyrus*, *Epistylis*, *Gyrodactylus*, *Ichthyobodo*, *Ichthyophthirius*, *Trichodina*, *Trichophrya*, *Argulus*, *Salmincola*, *Lernaea*, and *Ergasilus* in freshwater fish species when treated under a variety of rearing or environmental conditions. See Appendix VIa for a list of freshwater species authorized for treatment.

Objective B – Treatment of Ectoparasites in Marine Species

To determine the efficacy and safety of 35% PEROX-AID[®] treatment to control mortality caused by ectoparasites of the genera *Neobenedenia*, *Amyloodinium*, *Cryptocaryon*, and *Uronema* in marine fish species when treated under a variety of rearing or environmental conditions. See Appendix VIa for a list of marine species authorized for treatment.

VII. MATERIALS

A. Test and control articles:

a. Drug Identity

a. Active ingredient

Common Name: Hydrogen peroxide, Hydrogen peroxide for Aquaculture

Product Name: 35% PEROX-AID[®]

Chemical Name: H₂O₂, Dihydrogen dioxide, Hydrogen peroxide-35%

CAS Number: 7722-84-1

Appearance: Clear colorless liquid

Odor: slightly pungent odor

b. Strength and dosage form

Hydrogen peroxide is the active component of 35% PEROX-AID[®]. As formulated by the manufacturer, 35% PEROX-AID[®] contains 35% hydrogen peroxide w/w, and is used to control mortalities associated with external pathogens on fish or fish eggs. 35% PEROX-AID[®] is dissolved in water and applied as a static bath or flow-

through treatment. Treatments are administered at a specific concentration (based on the active ingredient) for up to 1 hour and then flushed from the fish-holding container.

c. Manufacturer, source of supply

Manufacturer:

Eka Chemicals, Inc.
1775 West Oak Commons Court
Marietta, GA 30062-2254
Contact Person: David Lovetro
Phone: 1-800-241-3900
Fax: 770-321-5865
Email: Dave.Lovetro@eka.com

Supplier:

Western Chemical, Inc.
1269 Lattimore Road
Ferndale, WA 98248-9424
Contact Person: Ron Malnor
Phone: 1-800-283-5292
Fax: 250-384-0270
ronm@wchemical.com

b. Verification of drug integrity/strength

The Manufacturer, Eka Chemical, Inc., will provide the analytical data necessary to establish the purity of each lot of 35% PEROX-AID[®] supplied. The lot number and date of expiration for each batch of 35% PEROX-AID[®] will be placed on the label of each container. The form "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (Form H₂O₂-1) will clearly identify the lot number of all 35% PEROX-AID[®] shipments. If the integrity of the 35% PEROX-AID[®] is compromised (i.e., by spilling or contamination of the stock container) it should not be used for treatment, and the event must be carefully recorded, dated, and signed in the Chemical Use Log (Form H₂O₂-2).

c. Storage Conditions

Ideally, 35% PEROX-AID[®] should be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. However, as it is possible that some facilities may have the need for both approved use of 35% PEROX-AID[®] (i.e., under NADA 141-255) and use under this INAD, it may be necessary for carefully measured aliquots of 35% PEROX-AID[®] to be transferred from "approved stock" and placed in a loosely-capped polyethylene plastic container and labeled specifically for INAD-use only. All 35% PEROX-AID[®] received, transferred and/or used for INAD-use should be carefully recorded on Form H₂O₂-2. To minimize the need for the transfer of 35% PEROX-AID[®] to auxiliary containers for INAD-use, it is strongly recommended that Investigators consider purchasing smaller quantities of 35% PEROX-AID[®] (i.e., 5 gallon containers vs 55 gallon containers) until such time as reliable INAD-use patterns are established. 35% PEROX-AID[®] should be stored away from direct sunlight, away from heat sparks or flames, and in a secure, cool, dry and properly vented location.

d. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for 35% PEROX-AID[®] (see Appendix IV). Each person involved with the study and each person who may be present during the use of 35% PEROX-AID[®] shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with 35% PEROX-AID[®].

e. Investigational labeling

Copies of the investigational labels to be attached to each container of 35% PEROX-AID[®] are provided in Appendix V. It is the responsibility of the Investigator to ensure proper investigational labeling of all containers of 35% PEROX-AID[®].

6. Accountability

- a. Western Chemical, Inc. will be the sole supplier of 35% PEROX-AID[®] to all Investigators under INAD 11-669.
- b. USFWS and Non-USFWS Facilities - Immediately upon receiving an order/shipment of 35% PEROX-AID[®], the Investigator will complete Form H₂O₂-1 "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The investigator will archive the original in the facility's INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form H₂O₂-1. The Study Monitor will then forward a copy to the Clinical Field Trial Coordinator at the Aquatic Animal Drug Approval Partnership Program. The Clinical Field Trial Coordinator will archive one copy, and send two copies of Form H₂O₂-1 to FDA. Arrangements should be made between Investigators and Study Monitors to ensure completed Form H₂O₂-1s are received by the Clinical Field Trial Coordinator in a timely manner.
- c. All Investigators are also responsible for maintaining an accurate inventory of 35% PEROX-AID[®] on-hand. A Chemical Use Log (Form H₂O₂-2) will be supplied to each Investigator. Each time 35% PEROX-AID[®] is used it must be recorded by the Investigator on Form H₂O₂-2.

7. Preparation Procedures

35% PEROX-AID[®] will be supplied to Investigators as an aqueous solution to be dissolved in culture water to achieve the required concentration based on active ingredient. Please note that 35% PEROX-AID[®] contains 35% hydrogen peroxide, w/w. Prior to actual use for treatment, a calculated and accurately measured amount of 35% PEROX-AID[®] (based on a pre-determined target treatment concentration) should first be mixed in a small volume of ambient temperature rearing water to establish a stock solution. After thorough mixing of 35% PEROX-AID[®], the stock solution should then be applied to, and thoroughly mixed with, rearing unit water. Consult the 35% PEROX-AID[®] label (Appendix Vb) for general directions for use. The product should not be adulterated in any manner prior to use.

B. Items needed for treatment, data collection, etc.:

Equipment and supplies needed should include items to sample fish, identify ectoparasites, and administer 35% PEROX-AID[®]. Sampling techniques and diagnostic equipment will most likely be provided by trained fish health biologists serving as Study Monitors or their designee(s).

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the 35% PEROX-AID[®] INAD will need to complete several forms. These forms are described in Section XIII (p 12). Copies of these forms are attached to this Study Protocol.

VIII. EXPERIMENTAL UNIT

The experimental unit in clinical field trials will consist of contained or isolated groups of fish. This will generally be groups of fish contained in tanks, raceways, or ponds. The

experimental unit will **not** be individual fish.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol before 35% PEROX-AID[®] can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (See Section XX). However, it is important to note that poor planning and/or lack of preparation will not be considered an emergency situation.

B. The characteristics of the study animals (species, size, number, etc.) are presented in Appendix VIb.

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of water temperatures and water quality parameters. Environmental conditions will be reported on Form H₂O₂-3.

D. Ability of Investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

E. Pathogen/disease considerations

1. Ectoparasites should be identified by procedures described in Chapter 3.1 General Procedures for Parasitology of the Fish Health Section Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, 2005 Edition, Fish Health Section/American Fisheries Society.
2. There should be increased mortality rates in rearing units for three or more consecutive days prior to initiation of treatment. However, station history and the experience of the Investigator, Study Monitor, or the fish health biologist may override this criterion to halt potentially explosive disease outbreaks. In such cases, however, careful diagnostic surveillance should be carried out in all rearing units proposed for treatment and controlled tests should be carried out if at all possible.
3. Typical disease signs should be detectable in at least a few fish and the causative ectoparasite should be identified.
4. Since the efficacy of 35% PEROX-AID[®] therapy for the control of ectoparasites is being tested, investigators must be prepared to make no changes in the fish cultural procedures or environmental conditions and apply no other treatments once a decision has been made to conduct 35% PEROX-AID[®] therapy. Concomitant or other aquatic pathogens should be carefully documented. If necessary, these pathogens can be treated once 35% PEROX-AID[®] response (efficacy) data has been collected. However, it may require as long as 10 days after the completion of 35% PEROX-AID[®] therapy to determine differences between test and control groups and to complete post-treatment evaluations.

Prior to initiating each treatment event, the Investigator must first complete Form H₂O₂-W, "Worksheet for Designing Individual Field Trials" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent by Fax to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward (Fax) the paperwork to the Aquatic Animal Drug Approval Partnership (AADAP) Program Office. The AADAP Office will then review the worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study

Monitor of the assigned number and approval to proceed. In most cases, this entire process can be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on Form H₂O₂-2 and Form H₂O₂-3, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, and infection/disease/treatment need is rapidly escalating, the Investigator should contact the AADAP Office for a study number and permission to proceed.

X. TREATMENT GROUPS

- A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish.
- B. Separately confined, untreated control fish will not be required in supportive field studies conducted to determine the effectiveness of 35% PEROX-AID[®] treatment. Fish from a group or lot will first be examined to determine if treatment with 35% PEROX-AID[®] is required. When treatment is underway or has been completed, fish from the same group will be examined to determine the effect of treatment on the parameters used to initiate the treatment. Evaluation will in all cases consist of determining fish mortality, and in most cases degree or severity of ectoparasite infestation will also be quantified.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals. Use of control groups will ensure that results of efficacy studies provide useful information that will support an NADA.

It is important that all fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Separating diseased or stressed fish into new groups for treatment may change environmental conditions responsible for the disease outbreak. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Although not required, replicate treatment groups are strongly encouraged in both treated and control groups. Assignment to control and treatment groups should be random and designed to avoid bias.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which treatment response observations are recorded by individuals who are unaware which fish have been treated and which fish are controls.

XI. TREATMENT SCHEDULES

- A. Route of administration
35% PEROX-AID[®] will be administered as either a static immersion or as a flow-through bath treatment.

B. Treatment dose/concentration, duration and interval

1. Objective A: To control mortality in freshwater finfish caused by external parasites of the genera *Ambiphrya*, *Chilodonella*, *Dactylogyrus*, *Epistylis*, *Gyrodactylus*, *Ichthyobodo*, *Ichthyophthirius*, *Trichodina*, *Trichophrya*, *Argulus*, *Salmincola*, *Lernaea*, and *Ergasilus*. See table below.

	Fresh Water Finfish ¹		
Dose/Concentration (mg/L)	100 or 150	50, 75 or 100	200 ²
Duration (min) per daily treatment	30	60	30
Total maximum number of treatments	3	3	3
Treatment interval (days)	consecutive or alternate	Consecutive or alternate	consecutive or alternate
<p>Footnotes: 1. Caution should always be exercised when treating a specific species/population for the first time. A small sub-sample of test fish should be treated first at the planned target dosage and planned treatment duration before treatment of an entire population or lot.</p> <p>2. Treatment at 200 mg/L is restricted to those sites where the investigator has demonstrated to the Study Monitor that treatment at lower concentrations were ineffective or when the investigator wishes to test multiple treatment concentrations simultaneously.</p>			

2. Objective B: To control mortality in marine finfish caused by ectoparasites of the genera *Neobenedenia*, *Amyloodinium*, *Cryptocaryon*, and *Uronema*. See table below.

	Marine Finfish ¹		
Dose/Concentration (mg/L)	100 or 150	50, 75 or 100	200 ²
Duration (min) per daily treatment	30	60	30
Total maximum number of treatments	3	3	3
Treatment interval (days)	consecutive or alternate	consecutive or alternate	consecutive or alternate
<p>Footnotes: 1. Caution should always be exercised when treating a specific species/population for the first time. A small sub-sample of test fish should be treated first at the planned target dosage and planned treatment duration before treatment of an entire population or lot.</p> <p>2. Treatment at 200 mg/L is restricted to those sites where the investigator has demonstrated to the Study Monitor that treatment at lower concentrations were ineffective or when the investigator wishes to test multiple treatment concentrations simultaneously.</p>			

3. For a static immersion bath treatment, 35% PEROX-AID[®] should be administered to the rearing unit at a specific concentration based on active ingredient. For a flow-through bath treatment, 35% PEROX-AID[®] should be administered into the incoming water supply at a flow rate adequate to achieve a specific treatment concentration based on active ingredient.
4. Within the parameters outlined above in Section XI.B., specific treatment concentration, treatment duration and dosing interval applied will be at the discretion of the Investigator.
5. After completion of treatment, either the treatment solution should be flushed from the rearing unit or the fish removed to fresh water.
6. Physical and biological variables such as age, fish species, water quality characteristics, environmental conditions, etc. may affect fish sensitivity to 35% PEROX-AID[®]. **Before conducting 35% PEROX-AID[®] treatments, Investigators are strongly encouraged to expose a small number of test fish to the treatment concentration before treating the entire group.**

C. Drug preparation and administration procedures

Standard personal protective equipment such as gloves, aprons, eye protection, etc. should be worn at all times when preparing or administering 35% PEROX-AID[®]. 35% PEROX-AID[®] for each individual lot of fish should be accurately measured volumetrically prior to treatment. To aid in the uniform distribution of chemical, 35% PEROX-AID[®] should be thoroughly mixed in a small volume of culture water to obtain a “stock solution” before application to rearing units. Remove dead fish and clean rearing units before application. The stock solution should then be thoroughly mixed with (or metered into) rearing water.

D. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other drug therapy once a decision has been made to conduct 35% PEROX-AID[®] treatment. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the 35% PEROX-AID[®] treatment involved should be appropriately labeled. If mortality continues at pretreatment levels following treatment and the causative agent is still present, the Study Monitor should be consulted.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or other pertinent species information indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form H₂O₂-3. Treatment response parameters that should be addressed include the following:

A. Primary Parameters

Morbidity and mortality data, coupled with case history and diagnosis of ectoparasites, usually indicate when 35% PEROX-AID[®] treatment is needed. Typically, **source data must be collected for at least 5 days before treatment, each day during the treatment period, and for at least 10 days after the**

treatment period has ended. Collection of these data are critically important in all cases. Gill, skin, fin, mucous or other tissue from groups of representative fish should be evaluated using appropriate methodology to determine ectoparasite presence and load.

B. Secondary Parameters

Secondary parameters should also include general observations on fish behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

C. Adverse Reactions

Any adverse reaction to treatment should be reported immediately to the Study Monitor, who will in turn notify the Study Director. Such responses might include changes in water quality, extremely negative responses/behavior by the fish, or hazards to the applicator. Although 35% PEROX-AID[®] has been used extensively with beneficial effect in fish culture, it is possible that adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions including apparent drug toxicity. Signs of drug toxicity should also be immediately reported to the Study Monitor, who will in turn notify the Study Director.

Note: Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the 35% PEROX-AID[®] INAD will need to complete the following forms:

- Form H₂O₂-W. Worksheet for Designing Individual Field Trials under 35% PEROX-AID[®] INAD 11-669
- Form H₂O₂-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
- Form H₂O₂-2. Chemical Use Log for Clinical Field Trials Using 35% PEROX-AID[®] under INAD 11-669
- Form H₂O₂-3. Results Report Form for Use of 35% PEROX-AID[®] under INAD 11-669

Copies of these forms are attached to this Study Protocol.

XIV. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data must be original, i.e., they must be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet must be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries must be properly attributed to each person. The data must be accurate and legible. If a mistake is made, it

must be crossed out using a single strike-through and the correct data must be recorded next to it. Each change to the raw data must be initialed and dated by the person making the change, and a statement must be provided explaining why the change was made. If the data sheet needs to be copied, all data must be transferred, including the properly noted changes. The original record must be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of according to standard hatchery practices. Although it is strongly recommended that all treated fish (both freshwater and marine species) be maintained at culture facilities for at least 10 days following final treatment before they are stocked or transferred to allow complete collection of efficacy trial data, treated fish may be allowed to enter the food chain immediately after treatment (i.e., 0-day withdrawal time) if such action is required to meet critical fishery management needs. This 0-day withdrawal time is consistent with the approved label for 35% PEROX-AID®.

The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form H₂O₂-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

35% PEROX-AID® will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or out-dated 35% PEROX-AID® remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. The investigational drug may not be redistributed to others not specified in the Study Protocol.

XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES

A. Drug distribution

See Section VII.A.6. Accountability (page 7) for information and details.

B. Study Monitors

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor should be assigned to each facility that is authorized to treat fish with 35% PEROX-AID®. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the 35% PEROX-AID® itself) are already available at each participating fish hatchery. The use of various drugs, chemicals, and therapeutants to meet management and/or production goals is a common occurrence at most fish hatcheries. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 7).

D. Administrator of the drug

35% PEROX-AID[®] will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). 35% PEROX-AID[®] will be maintained in a secure location, and only the Investigator or persons under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 7) for details and Forms H₂O₂-W, H₂O₂-1, H₂O₂-2, and H₂O₂-3 (page 12) for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitors who will review the information and ensure that all required data is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare an annual report that will be submitted to the FDA.

G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form H₂O₂-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. A copy of Form H₂O₂-2 should be sent to Study Monitors with the corresponding Form H₂O₂-3 (if no further treatments are necessary/planned), or at the end of the calendar year. A copy of Form H₂O₂-3 should be sent to the Study Monitor after completion of the entire treatment period, which includes the post-treatment observation period. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, Study Monitors should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the Aquatic Animal Drug Approval Partnership Program Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared and submitted to the FDA. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarded to the FDA for review. Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or in a manner different than specified in the Study Protocol, if forms are not filed on time,**

or if the study data are not properly collected, maintained, and reported. The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, what steps were taken to mitigate the deviation and the Investigator's opinion of the impact on study outcome.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with Form H₂O₂-3, and ultimately be submitted to the Study Director.

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