

**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, Fish and Aquatic Conservation

Sponsor Signature

Date Approved

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

Office 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: December 31, 2026
Study Director: Ms. Bonnie Johnson

Clinical Field Trial Location:

Facility: _____
Type or Print Name

Investigator: _____
Type or Print Name

Investigator Signature

Date

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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*, *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. **Note: No clinical field trials will be conducted under this INAD for use patterns for which 35% PEROX-AID® has already received FDA-approval (e.g., treatment of bacterial gill disease in freshwater-reared salmonids; treatment of external columnaris in freshwater-reared coolwater and warmwater finfish; treatment of saprolegniasis in freshwater-reared finfish eggs, freshwater-reared coldwater finfish, and fingerling and adult freshwater-reared cool- and warmwater finfish; and treatment of *Gyrodactylus* spp. in freshwater-reared salmonids (NADA 141-255)).**

II. SPONSOR

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Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

Study Director: Ms. Bonnie Johnson, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9905; Email: bonnie_johnson@fws.gov

Principal Clinical Field Trial Coordinator: Ms. Paige Maskill, USFWS – AADAP Program
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Phone: 406-994-9911; Email: paige_maskill@fws.gov

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: December 31, 2026

V. BACKGROUND/PURPOSE

A. 35% PEROX-AID® is currently approved in the United States for treatment of bacterial gill disease in freshwater-reared salmonids; treatment of external columnaris in freshwater-reared coolwater and warmwater finfish; treatment of saprolegniasis in freshwater-reared finfish eggs, freshwater-reared coldwater finfish, and fingerling and adult freshwater-reared cool- and warmwater finfish; and treatment of *Gyrodactylus* spp. in freshwater-reared salmonids (NADA 141-255). If your treatment is for an approved use then the INAD will not be used.

B. 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.

C. 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.

D. 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.

E. 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. **Note: no clinical field trials will be conducted under this INAD for use patterns for which 35% PEROX-AID® has already received FDA-approval (e.g., treatment of bacterial gill disease in freshwater-reared salmonids; treatment of external columnaris in freshwater-reared coolwater and warmwater finfish; treatment of saprolegniasis in freshwater-reared finfish eggs, freshwater-reared coldwater finfish, and fingerling and adult freshwater-reared cool- and warmwater finfish; and treatment of *Gyrodactylus* spp. in freshwater-reared salmonids (NADA 141-255)).**
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.

VII. MATERIALS

A. Test and control articles:

1. 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

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

2. Verification of Drug Integrity/Strength

The manufacturer 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). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. 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.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Safety Data Sheet (SDS) 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 SDS. Safety precautions as outlined in the SDS will be followed at all times when working with 35% PEROX-AID[®].

5. Investigational Labeling

A copy of the label to be attached to each container of 35% PEROX-AID[®] are provided in Appendix V. Although investigational labels will be affixed to 35% PEROX-AID[®] containers by the supplier, it is the responsibility of the Investigator to ensure proper labeling of all containers of 35% PEROX-AID[®].

6. Accountability

Syndel USA will be the sole supplier of 35% PEROX-AID[®] to all Investigators under INAD 11-669.

The Online INAD Database must be used by Investigators for ALL INAD reporting. The online INAD database has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting and accountability follows established INAD Study Protocol guidelines. Unless data is entered directly into the online INAD database (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the online INAD database) investigators must archive hard copies of all raw data.

1. All facilities using 35% PEROX-AID[®]:

Immediately upon receiving an order/shipment of 35% PEROX-AID[®], the Investigator must complete Form H2O2-1 “Report on Receipt of Drug -

Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (located in the "Manage/View Drug Inventory" section of the investigator account). The Study Director will forward a copy of this form to the FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form H2O2-1s are received by the Study Director within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of 35% PEROX-AID[®] on-hand. A Chemical Use Log (Form H2O2-2) must be completed and maintained by each Investigator. Each time 35% PEROX-AID[®] is used, it must be recorded by the Investigator in the Results Report form in the "Amount of Drug Used" table.

At the conclusion of the study, all remaining 35% PEROX-AID[®] will be destroyed by following the SDS (note: unless 35% PEROX-AID[®] is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). Disposition of all 35% PEROX-AID[®] must be properly recorded and accounted for in the Drug Inventory Form of the database which is Form H2O2 -2. The Study Monitor will be responsible for verifying the quantity of 35% PEROX-AID[®] remaining on hand versus the amount indicated on Form H2O2 -2. **Note:** 35% PEROX-AID[®] can be transferred to other facilities that are participating under INAD 11-669. Transfers must be shown in the Drug Inventory section of the database (formerly 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 located in the online INAD database. These forms are described in Section XIII. Copies of these forms are attached to this Study Protocol and will be used as a guide only for collecting the data that will be entered into the online INAD database.

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 for the current calendar year 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. Drug discharge must be in compliance with local **NPDES** permitting requirements.

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).

Prior to initiating each treatment event, the Investigator must first complete Form H2O2-W. “Worksheet for Designing Individual Field Trials” (located under the “New Study Request” tab in the investigator account) that pertains to each specific treatment event. The worksheet should be filled out and forwarded to the Study Monitor through the online INAD database. The Study Monitor will review the planned treatment (worksheet) and forward it to the Study Director at the AADAP Office. The Study Director will then review the worksheet, assign the approved treatment a Study Number, and then the online INAD database will notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on any paper forms that are being used as a guide to collect the data to enter in the online database (i.e., Form H2O2-2 and H2O2-3), as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach the Study

Monitor with regards to Worksheet approval and the need for treatment is immediate, the Investigator should contact the AADAP Office for permission to proceed.

Note: The online INAD database, which must be used by Investigators for all INAD reporting, has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting follows established INAD Study Protocol guidelines.

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.

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 supplementary field studies conducted to determine the effectiveness and safety of 35% PEROX-AID® immersion therapy. 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 initially sanction the treatment. Evaluation will in all cases consist of determining fish mortality, although in some cases degree or severity of ectoparasite infestation may also be quantified.
- C. Although as stated above untreated control groups are not a required element of treatment under this INAD exemption, **it is important for all investigators to note that field trials conducted under a more stringent study protocol (i.e including requirements for non-treated controls groups, replication, blinding, dose verification, etc.) will ultimately be required in order to support a NADA for 35% PEROX-AID®. It is also important to note that the INAD sponsor fully expects that a limited number of facilities/investigators listed under this INAD exemption will agree to participate in such “pivotal” efficacy studies.** These studies will be initiated only after direct consultation between facilities/investigators and the sponsor. These studies will be conducted under a separate FDA-approved study protocol (i.e. not the INAD study protocol), and will also be conducted with assistance from, and under the direct supervision of, the sponsor. **If for any reason it becomes**

apparent to the sponsor that facilities/investigators listed under this INAD are not willing to participate in such “pivotal” studies, the sponsor will request that FDA terminate the INAD.

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*, *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

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.
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.

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
Footnotes:	<p>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. Objective C: 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)	400
Duration (min) per daily treatment	45
Total maximum number of treatments	1
Treatment interval (days)	Once per week (minimum of 7 days between treatments)
Footnotes:	<p>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>

- a. 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.
- b. 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.
- c. After completion of treatment, either the treatment solution should be flushed from the rearing unit or the fish removed to fresh water.
- d. 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 minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with 35% PEROX-AID®.

However, if concomitant therapy is required in order to protect valuable fish stocks (i.e., threatened and endangered species not for human consumption) it should be fully documented and the efficacy data from the 35% PEROX-AID® treatment involved should be appropriately labeled. Contact the AADAP Office for the information that will need to be provided in the Form H2O2-3 if concomitant therapy is conducted.

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:

1. Primary Parameters

Morbidity and mortality data, coupled with case history and diagnosis of ectoparasites, usually indicate when 35% PEROX-AID[®] treatment is needed. **Source data must be collected for 5 days before treatment, during treatment, and for 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 (or absence) and load.

2. 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.

3. 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. **If any signs of drug toxicity are detected, they should also be documented and 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 35% PEROX-AID® INAD 11-669 will need to complete the following forms:

- Form H2O2-W. Worksheet for Designing Individual Field Trials under 35% PEROX-AID® INAD 11-669 - located in the New Study Request tab
- Form H2O2-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals – located in the Manage/View Drug Inventory tab
- Form H2O2-2. Chemical Use Log for Clinical Field Trials under 35% PEROX-AID® INAD 11-669 – located in the Manage/View Drug Inventory tab and filled out in Form H2O2-3 to show use
- Form H2O2-3. Results Report Form for Use of 35% PEROX-AID® INAD 11-669 – located in the Active Studies table on the home page

Copies of these forms are attached to this Study Protocol. Actual reporting is accomplished on forms located in the online INAD database.

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located in the online INAD database. All current and completed studies conducted under the investigator account will be stored and available in the online INAD database to the current study monitor, study investigator, and AADAP.

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 35% PEROX-AID® remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. Drug disposal information is available in the Safety Data Sheet (SDS) located in Appendix IV of this protocol. Disposition of all 35% PEROX-AID® must be properly recorded and accounted for on the Chemical Use Log (Form H2O2-2). The Study Monitor will be responsible for verifying the quantity of 35% PEROX-AID® remaining on hand versus the amount indicated on Form H2O2-2. The investigational drug may not be redistributed to others not specified by the protocol and should not be retained by the Investigator after completion of the study (note: unless 35% PEROX-AID® is planned for use in another approved field trial, and planned usage is within the storage guidelines established by the manufacturer). The investigational drug may not be redistributed to others not specified in the Study Protocol. Transfers must be shown on Form H2O2-2.

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

A. Drug distribution

See Section VII.A.6. Accountability 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 will be selected by each facility that is authorized to treat fish with 35% PEROX-AID® under this INAD. 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.).

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. See Section VII.A.6. Accountability for details and the following forms will be used as guides for data collection: Form H2O2-W, Form H2O2-1, Form H2O2-2, and Form H2O2-3.

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 Monitor who will ensure that all required information 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 summary reports that will be submitted to the FDA. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

G. Data storage

The Investigator is responsible for complete and accurate data collection, and must complete all required data forms (see protocol Section XIII). The Investigator should forward all completed forms to the Study Monitor for review. Study Monitors should carefully check each set of data for accuracy and completeness. If a form is incomplete or inaccurate, it should be returned to the Investigator. If a form is complete and accurate, it should be forwarded to the Study Director at the AADAP Office. **Note:** data that is entered through the online INAD database will be archived in the database. These archived forms will be available as long as the study participant accounts remain open.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. INAD reports will be prepared and submitted to the FDA as required. 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 a field trial 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 forwarder 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, and what steps were taken to mitigate the deviation.** Deviations should be documented on Form H2O2-3 in the *Description of Results* section and in the *Study Deviation* field.

XXI: E.O. 13891

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency policies.

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Appendix I. Sponsor Contact Information for 35% PEROX-AID® INAD #11-669

Sponsor: Dr. Marilyn Blair, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9904
Fax: (406) 582-0242
Email: marilyn_j_blair@fws.gov

Sponsor Address: 4050 Bridger Canyon Road, Bozeman, MT 59715

Study Director: Ms. Bonnie Johnson
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9905
Fax: (406) 582-0242
Email: bonnie_johnson@fws.gov

**Principal Clinical Field
Trial Coordinator:** Ms. Paige Maskill
Aquatic Animal Drug Approval Partnership (AADAP) Program
Phone: (406) 994-9911
Fax: (406) 582-0242
Email: paige_maskill@fws.gov

Appendix II. Study Monitors for 35% PEROX-AID[®] INAD #11-669

Note: This information will be provided directly to CVM

Appendix IIIa. Facilities and Names of Investigators Participating under 35% PEROX-AID® INAD #11-669

Note: This information will be provided directly to CVM and Syndel

Appendix IIIb. Sample of Knowledge Required for Position of Hatchery Manager (i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal, State, Tribal, and private agencies/facilities.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.

Appendix IV. Safety Data Sheet (SDS) for 35% PEROX-AID® INAD #11-669

The SDS for 35% PEROX-AID® can be found at the drug sponsors website

http://www.syndel.com/downloads/dl/file/id/10/35_perox_aid_sds.pdf

Appendix V. Investigational Label for 35% PEROX-AID® INAD #11-669

1. Investigational label for tests in vitro and in laboratory research animals [511.1(a)]:

"Caution. Contains a new animal drug for investigational use only in laboratory animals or for tests in vitro. Not for use in humans."

2. Investigational label for use in clinical field trials [511.1(b)]:

"Caution. Contains a new animal drug for use only in investigational animals in clinical field trials. Not for use in humans. Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture."

Appendix VIa. Fish Species Treated under 35% PEROX-AID® INAD #11-669

Freshwater finfish
Saltwater finfish

Appendix VIb. Table of Facilities and Fish Stocks Treated under 35% PEROX-AID® INAD #11-669

Note: This information will be provided directly to CVM

All data must be entered through the online INAD database:

The following forms are to be used as a guide for collecting data that will be entered into the **online INAD database**. Any paper forms that are submitted to AADAP will be sent back to the study participants.

Form H2O2-W: Worksheet for Designing Individual Field Trials Under 35% PEROX-AID® INAD 11-669

INSTRUCTIONS

1. Investigator must fill out Form H2O2-W for each trial conducted under this INAD before actual use of 35% PEROX-AID®.
2. Investigator should forward a copy of H2O2-W to the Study Monitor for review.
3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of the Study Number.

SITE INFORMATION

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Fax	

FISH CULTURE AND DRUG TREATMENT INFORMATION

Pathogen type	Ectoparasite		
	Study Objective A or B (circle one)	Objective A Freshwater fish species	Objective B Marine fish species
Fish species to be treated		Ectoparasite to be treated	
Average fish weight (gm)		Average fish length (in)	
Number of fish per rearing unit (i.e., tank, raceway, or pond)		Number of rearing units to be treated	
Total number of fish to be treated		Number of control rearing units/number of control fish	/
Intended hydrogen peroxide (35% PEROX-AID®) dosage (mg/L)		Planned duration of treatment (minutes)	
Planned number of treatments		Treatment on consecutive or alternate	
Estimated amount of 35% PEROX-AID needed for treatment (L)			
Anticipated date treatment will be initiated			

STUDY DESIGN: Provide a brief description of your planned study. The description should include the reason you feel fish should be treated, the treatment dates, the number of fish that will be treated, and if the fish are a threatened or endangered species.

Study designed by; _____

DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in the Study Protocol.

USE AND DISPOSITION OF HYDROGEN PEROXIDE (35% PEROX-AID®)
(Environmental Safety Considerations):

Investigator should initial here to indicate awareness that hydrogen peroxide (35% PEROX-AID®) usage and disposition must be in compliance with requirements described in the Study Protocol.

WORKER SAFETY CONSIDERATIONS:

Investigator should initial here to indicate that all personnel handling hydrogen peroxide (35% PEROX-AID®) have read the Material Safety Data Sheet for hydrogen peroxide (35% PEROX-AID®) and have been provided personal protective equipment, in good working condition, as described in the Study Protocol.

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

FORM H2O2-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

INSTRUCTIONS

- Investigator must fill out Form H2O2-1 immediately upon receipt of Reward®.
- Investigator should forward a copy of Form H2O2-1 to the Study Director at the AADAP Office.

The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act.

Name of Drug	Hydrogen peroxide (35% PEROX-AID®)	INAD Number	XX-XXX
Proposed Use of Drug	Treatment of ectoparasites that occur in a variety of freshwater and marine finfish		
Date of CVM Authorization Letter	5/26/2021		
Date of Drug Receipt		Amount of Drug Received	
Drug Lot Number		Trial Number	
Name of Investigator			
Address of Investigator			
Location of Trial			
Pivotal Study	Yes	Non-pivotal Study	----
Approximate Number of Treated Animals		Approximate Number of Control Animals	
Number of Animals Used Previously ¹			
Study Protocol Number	11-669		
Approximate dates of trial (start/end)			
Species, Size, and Type of Animals			
Maximum daily dose and duration	400mg/L for 45 minutes; 200 mg/L for 30 minutes; 100 mg/L for 60 minutes		
Methods(s) of Administration	Immersion (static or flow-through treatment)		
Withdrawal Period	0-day; all species		

¹ To be filled out by the AADAP Office

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____

Date Reviewed: _____

Sponsor: _____

STUDY NUMBER _____

Form H2O2-3: Results Report Form for Use of 35% PEROX-AID® Under INAD 11-669

INSTRUCTIONS

1. Investigator must fill out Form H2O2-3 no later than 30 days after completion of the study period. Attach lab reports and other pertinent information.
2. If 35% PEROX-AID® was not used under the assigned Study Number, contact the Study Director at the AADAP Office on how to close-out the study.
3. Investigator should forward a copy of Form H2O2-3 to the Study Monitor. Within 10 days of receipt, the Study Monitor should forward a copy to the Study Director at the AADAP Office.

SITE INFORMATION

Facility	
Reporting Individual	

FISH CULTURE AND DRUG TREATMENT INFORMATION

35% PEROX-AID® lot number		Total amount of drug used (L)	
Study Objective A or B (circle one)		<u>Objective A</u> Freshwater fish species	<u>Objective B</u> Marine fish species
Fish species to be treated		Ectoparasite to be treated	
Average fish weight (gm)		Average fish length (in)	
Number of fish per rearing unit (i.e., tank, raceway, or pond)		Number of treated rearing units	
Total number of fish to be treated		Number of control rearing units/number of control fish	/
35% PEROX-AID® dosage used (mg/L)		Treatment duration (minutes)	
Number of treatments		Treatment on alternate or consecutive days	
Treatment date(s)			

WATER QUALITY PARAMETERS

Ave treatment temp (°F)		Dissolved Oxygen (mg/L)	
pH		Hardness - CaCO ₃ (mg/L)	

Daily Mortality Record

INSTRUCTIONS

1. Investigator should fill out the Daily Mortality Record as completely as possible.
2. Prior to initiation of the trial, fill out Rearing Unit ID, whether a rearing unit is Treated or Control, and the number of fish in each rearing unit.
3. Water temperature and individual tank mortality should be recorded on a daily basis.
4. If treatment is on 3 consecutive days, fill in only days 1-3 of the “treatment period” and proceed directly to day 1 of the “post-treatment period”. If less than 3 treatments are used, proceed directly to day 1 of the “post-treatment period” after the final treatment. Please mark all treatment days with an asterisk.
5. **Even if mortality is zero an entry is still needed for that day.**

FACILITY										
	Rearing Unit ID									
	Treated or Control									
	Number of Fish									
	Day	Date	Water Temp (F°)	Mortality	Mortality	Mortality	Mortality	Mortality	Mortality	Daily Observer Initials
Pre-Treatment Period	1									
	2									
	3									
	4									
	5									
Treatment Period	1									
	2									
	3									
	4									
	5									
Post-treatment Period	1									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									

STUDY NUMBER _____

RESULTS: Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Describe general fish behavior, including feeding behavior. Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol?

PATHOLOGY REPORT: Attach pathology report to this form. Report should include: 1) a description of how the pathogen(s) was identified; 2) disease identification records that confirm the presence of the pathogen; and 3) the name and title of the individual performing the diagnosis.

Pathology Report included: _____ pre-treatment _____ post-treatment

TOXICITY OBSERVATIONS: Report any apparent drug toxicity including a description of unusual fish behavior.

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

Investigator should initial here to indicated awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Study Protocol Section XV

Estimated number of days between last treatment and first availability of fish for human consumption (ensure this time period meets the withdrawal period).

DISPOSITION OF HYDROGEN PEROXIDE (35% PEROX-AID®)

Use and disposition of all hydrogen peroxide (35% PEROX-AID®) followed Study Protocol guidelines and has been clearly identified on Form H₂O₂-2 (Investigator should initial)

NEGATIVE REPORT Hydrogen peroxide (35% PEROX-AID®) was not used at this facility under this Study Number during the reporting period. The study will be closed out in the online INAD database.

Date Prepared: _____

Investigator: _____

Date Reviewed: _____

Study Monitor: _____