Date of Approval: July 26, 2019

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-255

35% PEROX-AID®

hydrogen peroxide

Concentrated Immersion Solution

Freshwater-Reared Finfish

For the control of mortality in freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae

For the treatment and control of *Gyrodactylus* spp. in freshwater-reared salmonids

For the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*

Sponsored by:

Syndel USA

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I. GENERAL INFORMATION

A. File Number

NADA 141-255

B. Sponsor

Syndel USA
1441 W. Smith Rd.
Ferndale, WA 98248

Drug Labeler Code: 050378

C. Proprietary Name

35% PEROX-AID®

D. Drug Product Established Name

Hydrogen peroxide

E. Pharmacological Category

External microbicide

F. Dosage Form

Concentrated immersion solution

G. Amount of Active Ingredient

396.1 mg hydrogen peroxide/ml

H. How Supplied

5-gallon container (19L), 55-gallon drum (208L), and 328-gallon container (1240L)

I. Dispensing Status

Over-the-counter (OTC)

J. Dosage Regimen

See Indications section below

K. Route of Administration

Immersion

L. Species/Class

Freshwater-reared finfish

M. Indications

For the control of mortality in freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae: 75 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

For the treatment and control of *Gyrodactylus* spp. in freshwater-reared salmonids: 50 mg/L for 60 minutes, or 100 mg/L for 30 minutes, per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

For the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*: For fry, 50 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath. For fingerling and adult fish, 50-75 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

N. Effect of Supplement

This supplement provides for the addition of the following indications:

For the control of mortality in freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae.

For the treatment and control of *Gyrodactylus* spp. in freshwater-reared salmonids.

For the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*.

II. EFFECTIVENESS

Saprolegniasis: Three induced infection model studies were conducted to demonstrate the effectiveness of 35% PEROX-AID® for the control of mortality in freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae. These studies demonstrated that 35% PEROX-AID® is effective for this use based on studies where mortality associated with two different species of *Saprolegnia* was controlled in three different species of fish: rainbow trout, walleye, and channel catfish.

Gyrodactylus spp.: Four field studies were conducted to demonstrate the effectiveness of 35% PEROX-AID® for the treatment of *Gyrodactylus* spp. infestations in freshwater-reared salmonids. Three studies were conducted according to Good Clinical Practice (GCP); the fourth was missing several aspects of GCP but was of high enough quality to provide supportive evidence for effectiveness. Although all the studies involved the treatment of a single parasite species

(*Gyrodactylus salmonis*), additional information was provided that allowed the indication to include the treatment of *Gyrodactylus* spp. as a genus level group. A parasite survey conducted in 2011 showed that *G. salmonis* was the only species identified in *Gyrodactylus* samples obtained from five different salmonid species at 23 fish culture facilities in seven different states. Existing literature reports also indicate that *G. salmonis* is the most prevalent and most pathogenic *Gyrodactylus* species found on freshwater reared salmonids raised in the United States. Therefore, *G. salmonis* is considered to be representative of *Gyrodactylus* spp. in US freshwater-reared salmonids. Additionally, the non-specific nature of hydrogen peroxide treatment, physiological similarities among gyrodactylids, and literature reports of hydrogen peroxide sensitivity in other *Gyrodactylus* species provided support for the genus level claim and the likelihood of similar effectiveness among untested *Gyrodactylus* species. The World Organization for Animal Health (OIE) reportable pathogen, *G. salaris* is not known to be present in the United States.

Columnaris disease: 35% PEROX-AID® was previously approved for the control of mortality in freshwater-reared channel catfish (a warmwater finfish species) due to external columnaris disease associated with *Flavobacterium columnare* when administered at 50 mg/L for fry, and 50 to 75 mg/L for fingerling and adults, for 60 minutes per day on three alternate days. Two additional clinical field trials were conducted in largemouth bass and bluegill to extend this indication to all freshwater-reared warmwater finfish.

Effectiveness studies for all three indications are contained in the publicly disclosable INAD files 010023, sponsored by the U.S. Geological Survey, Upper Midwest Environmental Sciences Center; and 011669, sponsored by the U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership. They are also summarized in Public Master File 005639.

A. Dosage Characterization

This supplemental approval does not change the previously approved dosage range. The Freedom of Information (FOI) Summary for the original approval of NADA 141-255 dated January 11, 2007, contains dosage characterization information for use of the drug in finfish.

B. Substantial Evidence

1. Induced Infection Model Study

   Title: The Efficacy of Hydrogen Peroxide to Control Mortalities Associated with Saprolegniasis Infections on Channel Catfish. Study number CAP-03-H202-6.

   Study Dates: April 1, 2003, to May 18, 2003

   Study Location: La Crosse, Wisconsin, U.S.A.

   Study Design:

   Objective: To evaluate the effectiveness of 75 mg/L hydrogen peroxide as an immersion agent to control mortality due to *Saprolegnia parasitica* infection

on channel catfish. This study was conducted in general accordance with the Good Laboratory Practice regulations (21 CFR 58).

Study Animals: Juvenile catfish, approximately nine months old were selected for the study. Mean weight of the catfish was 5.6 g.

Experimental Design: Fish were anesthetized and a section of the epidermis and dermis was abraded. Fish were then exposed to *S. parasitica* for 6 hours. After immersion challenge, 30 fish per treatment group were randomly distributed to one of six treatment tanks (10 fish per tank). Test tanks were randomly assigned to one of the treatment groups (0 or 75 mg/L hydrogen peroxide). The individual tank was the experimental unit.

Drug Administration: Treatments of 0 or 75 mg/L hydrogen peroxide were administered once daily every other day for a total of 3 treatments. Each treatment lasted one hour. Tank water was stirred with a glass rod to ensure adequate mixing. Water flow was turned off for the duration of the 60 minute static immersion bath. Aeration of tests tanks continued during the static bath to maintain dissolved oxygen concentration at ≥ 60% saturation. Post-treatment, approximately 80% of the treatment water was removed and water flow resumed at 600 mL/min for at least 30 minutes, before being reduced to 300 mL/min for the remainder of the study.

Measurements and Observations: Cumulative mortality was the primary variable measured. Each tank was checked daily for mortalities during the dosing and post-dosing period (15 days total). Mortalities were removed from the test tank and the number of mortalities was recorded each day. Dead and moribund fish were visually observed for the presence of fungus. Fungus from dead fish was observed under a microscope to determine the presence of long branched non-septate hyphae and zoosporangia, characteristic of *S. parasitica*. Water samples were collected prior to the termination of each treatment to determine hydrogen peroxide concentration using a permanganate titration method. Temperature, dissolved oxygen, and pH were measured daily.

Statistical Methods: Cumulative tank mortality was analyzed using a generalized linear model with treatment as a fixed effect. Analysis was conducted assuming a binomial distribution and a logit link.

Results: Cumulative mortality results are included in Table 1. The cumulative percent mortality was statistically significantly different for catfish treated with 75 mg/L (one-sided $P < 0.025$) hydrogen peroxide compared to the non-treated controls.

Table 1: Mortality results for challenge study in catfish with a 3 alternate day treatment period and 10-day post-treatment period.

Hydrogen Peroxide Dose (mg /L)	Percent Cumulative Mortality
0	87 (26/30)
75	37 (11/30)

Visual observation showed fungus present on all mortalities and moribund fish. Microscopic observations were consistent with *S. parasitica* infections.

The mean dissolved oxygen was 10.2 mg/L. The mean pH was 7.72. The mean water temperature was 13.2 ºC. The water quality results were acceptable for the culture of catfish.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentration of 75 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: The results of this study demonstrate the effectiveness of hydrogen peroxide applied at 75 mg/L for 60 minutes every-other-day for three treatments for the control of mortality from *Saprolegnia parasitica* on channel catfish.

2. Induced Infection Model Study

Title: Efficacy of 35% PEROX-AID® to control mortality caused by *Saprolegnia parasitica* and *Saprolegnia diclina* in rainbow trout *Oncorhynchus mykiss*. Study Number AEH-08-PEROXAID-01.

Study Dates: February 26, 2009, to March 20, 2009

Study Location: La Crosse, Wisconsin, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of 35% hydrogen peroxide to control mortality due to saprolegniasis in juvenile rainbow trout following immersion challenge with *Saprolegnia parasitica* or *S. diclina*. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

Study Animals: Approximately 750 juvenile rainbow trout were selected for the study. Rainbow trout weighed and measured an average of 20.8 g and 12.2 cm, respectively.

Experimental Design: Subgroups of 50 fish were anesthetized and the epidermis and dermis were abraded. Fish were transferred to a static immersion challenge bath with either *S. parasitica* or *S. diclina* (approximately 360 fish for each bath). Fifteen (15) fish were randomly assigned to one of 18 test tanks after immersion challenge designated as

Banks A/B (*S. parasitica* challenge) and Banks C/D (*S. diclina* challenge). Test tanks were randomly assigned to a nontreated control group or one of two hydrogen peroxide treatment groups (50 or 75 mg/L hydrogen peroxide). Each tank was considered to be an experimental unit and there were six test tanks per treatment.

Drug Administration: Treatments of 0, 50, and 75 mg/L hydrogen peroxide were administered once daily every other day starting two hours after exposure ended, for a total of 3 treatments. Each treatment lasted one hour. Tank water was stirred with a glass rod to ensure adequate mixing. Water flow was turned off for the duration of the 60 minute static immersion bath. Aeration of tests tanks continued during the static bath to maintain dissolved oxygen concentration at ≥ 60% saturation. Post-treatment, approximately two-thirds of the treatment water was removed and water flow resumed at 600 mL/min.

Measurements and Observations: Cumulative mortality was the primary variable measured. Each tank was checked daily for mortalities during the dosing and post-dosing period (19 days total). Mortalities were removed from the test tank and the number of mortalities was recorded each day. Fungal culture results from mortalities were recorded. Fungal infection was confirmed by the presence of fungal growth on YPS (yeast, peptone, sucrose) agar plates.

Dissolved oxygen and pH were measured in six tanks, 10 minutes prior to the end of treatment on treatment days and daily on nontreatment days. Temperature and flow rate were measured in all tanks 10 minutes prior to the end of treatment on treatment administration days, and daily on non-treatment days.

Statistical Methods: Cumulative tank mortality at 14 days post-treatment was analyzed using a generalized linear model with treatment as a fixed effect. The analysis incorporated overdispersion and was performed assuming a binomial distribution and using a logit link. The mortality rate of each challenged treated group was individually compared to the mortality rate of the challenged control group using a two-sided means comparison test at 0.05 level of significance.

Results: The *S. parasitica* challenge induced 68.9% cumulative mortality in the non-treated control group. Cumulative percent mortality was statistically significantly different and reduced for rainbow trout treated with 50 ($P < 0.001$) and 75 mg/L ($P < 0.001$) hydrogen peroxide compared to the non-treated controls. Results for the *S. parasitica* challenge study are included in Table 2. The *S. diclina* challenge resulted in only 5.6% cumulative mortality in the non-treated control group. As the overall level of disease induction was insufficient to allow for any valid assessment of drug effectiveness, results from the *S. diclina* portion of the study are not included here.

Table 2: Mortality results for a *Saprolegnia parasitica* challenge study in rainbow trout treated with a 3 alternate day treatment period and 14-day post-treatment period.

Hydrogen Peroxide Dose (mg /L)	Percent Cumulative Mortality
0	68.9 (62/90)
50	33.3 (30/90)
75	31.1 (28/90)

All fish cultured were positive for *S. parasitica* (Banks A/B) and *S. diclina* (Banks C/D).

The mean dissolved oxygen was 10.1 mg/L. The mean pH was 7.33. The mean water temperature of the experimental tanks was 13.3 ºC. The water quality results were acceptable for the culture of rainbow trout.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentrations of 50 and 75 mg/L.

Adverse Reactions: During the first treatment, fish in the *S. parasitica* and *S. diclina* challenge groups that were treated with hydrogen peroxide exhibited typical signs of stress including loss of equilibrium and rising to the water surface. Signs resolved with water flow resumption to the tanks; there were no mortalities. This reaction was likely due to drug exposure in combination with the additional stressors of skin abrasion, pathogen challenge and general handling. No other adverse reactions occurred in subsequent treatments nor in any other treatments of rainbow trout at these concentrations.

Conclusions: The results of this study demonstrate the effectiveness of hydrogen peroxide applied at 50 and 75 mg/L for 60 minutes administered every other day for three treatments for the control of mortality from *Saprolegnia parasitica* on rainbow trout.

3. Induced Infection Model Study

Title: Efficacy of 35% PEROX-AID® to control mortality caused by *Saprolegnia parasitica* or *Saprolegnia diclina* in walleye *Sander vitreum*. Study Number AEH-08-PEROXAID-03.

Study Dates: November 29, 2010, to December 17, 2010

Study Location: La Crosse, Wisconsin, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of 35% hydrogen peroxide to control mortality due to saprolegniasis in juvenile walleye following immersion

challenge with *Saprolegnia parasitica* or *S. diclina*. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

Study Animals: Approximately 750 juvenile walleye were selected for the study. Walleye weighed and measured an average of 13.71 g and 11.6 cm, respectively.

Experimental Design: The fish were anesthetized with tricaine methanesulfonate and a section of the epidermis and dermis was abraded. Fish were transferred to a static immersion challenge bath with either *S. parasitica* or *S. diclina* (approximately 360 fish for each bath). Twenty (20) fish were randomly assigned to one of 18 test tanks after immersion challenge designated as Banks A/B (*S. parasitica* challenge) and Banks C/D (*S. diclina* challenge). Test tanks were randomly assigned to a nontreated control group or one of two hydrogen peroxide treatment groups (50 or 75 mg/L hydrogen peroxide). Each tank was considered to be an experimental unit and there were six test tanks per treatment group.

Drug Administration: Treatments of 0, 50, and 75 mg/L hydrogen peroxide were administered once daily every other day starting two hours after exposure ended for a total of 3 treatments. Each treatment lasted one hour. Tank water was stirred with a glass rod to ensure adequate mixing. Water flow was turned off for the duration of the 60 minute static immersion bath. Aeration of tests tanks continued during the static bath to maintain dissolved oxygen concentration at ≥ 60% saturation. Post-treatment, approximately two-thirds of the treatment water was removed and water flow resumed at 600 mL/min.

Measurements and Observations: Cumulative mortality was the primary variable measured. Each tank was checked daily for mortalities during the dosing and post-dosing period (19 days total). Mortalities were removed from the test tank and the number of mortalities was recorded each day. Fungal culture results from mortalities were recorded. Fungal infection was confirmed by the presence of fungal growth on YPS (yeast, peptone, sucrose) agar plates.

Water samples were collected prior to the termination of each treatment in order to determine hydrogen peroxide concentration using a permanganate titration method.

Dissolved oxygen and pH were measured in six tanks, 10 minutes prior to the end of treatment on treatment days and daily on non-treatment days. Temperature and flow rate were measured in all tanks 10 minutes prior to the end of treatment on treatment administration days, and daily on non-treatment days.

Statistical Methods: A generalized linear model which included the fixed effect of treatment was used to analyze cumulative mortality 14 days post-treatment period. The analysis incorporated overdispersion and was performed assuming a binomial distribution and using a logit link. The mortality rate of each challenged treated group was individually compared to

the mortality rate of the challenged control group using a two-sided means comparison test at 0.05 level of significance.

Results: The *S. diclina* challenge induced 86.3% cumulative mortality in the non-treated control group. Cumulative percent mortality was statistically significantly different and reduced for walleye treated with 50 mg/L ($P < 0.05$) and 75 mg/L ($P = 0.05$) hydrogen peroxide compared to the non-treated controls. Results for the *S. diclina* challenge study are included in Table 3. The *S. parasitica* challenge resulted in only 11.4% cumulative mortality in the non-treated control group. As the overall level of disease induction was insufficient to allow for any valid assessment of drug effectiveness, results from the *S. parasitica* trial are not included here.

Table 3: Mortality results for *Saprolegnia diclina* challenge study in walleye treated every other day for three treatments, with a 14-day post-treatment period.

Hydrogen Peroxide Dose (mg /L)	Percent Cumulative Mortality
0	86.3 (98/114)
50	68.8 (87/126)
75	70.8 (85/120)

All fish cultured were positive for *S. parasitica* (Banks A/B) and *S. diclina* (Banks C/D).

The mean dissolved oxygen was 10.25 mg/L. The mean pH was 8.20. The mean water temperature of the experimental tanks was 12.8 ºC. The water quality results were acceptable for the culture of walleye.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentrations of 50 and 75 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: The results of this study demonstrate the effectiveness of hydrogen peroxide applied at 50 and 75 mg/L for 60 minutes administered every other day for three treatments for the control of mortality from *Saprolegnia diclina* on walleye.

4. Field Effectiveness Study

Title: Field effectiveness of 35% PEROX-AID® (hydrogen peroxide) to reduce *Gyrodactylus* spp. infestation density in lake trout *Salvelinus namaycush*. Study number AEH-10-PEROXAID-01.

Study Dates: August 23, 2010, to September 11, 2010

Study Location: Iron River, Wisconsin, U.S.A.

Study Design:

Objective: To confirm the efficacy of 35% PEROX-AID® in reducing *Gyrodactylus* spp. infestation density on lake trout, *Salvelinus namaycush*. This study was conducted in accordance with Good Clinical Practice.

Study Animals: 1,170 juvenile lake trout; mean length: 6.9 cm, mean weight: 2.99 g.

Experimental Design: 18 tanks containing 65 fish each were divided into two blocks of nine tanks. Within each block, tanks were randomly assigned to a nontreated control group or one of two hydrogen peroxide treatment groups (50 or 100 mg/L hydrogen peroxide). Each tank was considered to be an experimental unit and there were six test tanks per treatment group.

Drug Administration: Fish in treated tanks were treated with hydrogen peroxide as a static bath at a dosage of 50 mg/L for 60 minutes per day or 100 mg/L for 30 minutes per day, on three alternate days. Fish in control tanks received a sham treatment of water.

Measurements and Observations: Two weeks prior to treatment, the fish in the reference population underwent a U.S. Fish and Wildlife Service fish health inspection to assess the general health of the population. Immediately before enrollment, skin scrapes were taken from ten fish in the reference population and all *Gyrodactylus* present in the samples were counted and recorded. The primary response variable was *Gyrodactylus* abundance (count) on post-treatment day 14. Abundance was also assessed on post-treatment day 1. Two fish were indiscriminately selected from each tank for parasite evaluations on post-treatment day 1. On post-treatment day 14, five fish were indiscriminately selected from each tank for parasite evaluations. The fish were removed from the population and all *Gyrodactylus* found on their head, gills, and caudal fin were counted and recorded. Water quality and mortality were monitored. Concentration of hydrogen peroxide was verified from water samples collected from each test tank on each treatment day.

Statistical Methods: A generalized linear mixed model with a log link and an overdispersion parameter was used to separately analyze parasite abundance at 1 and 14 days post-dosing. The model included treatment as a fixed effect, and block and tank nested within block-by-treatment interaction as two random effects.

The treatment and control group means were estimated using back-transformed least squares mean estimates, and percent effectiveness was computed using the following formula:

percent effectiveness =
100 × (1 – geometric mean treated/geometric mean control)%

The primary variable was parasite infestation density at day 14 after the last treatment. A treatment regimen was considered effective if it satisfied the following criteria: (1) a statistically significant difference between the treated

and control groups at two-sided α=.05, and (2) the percent effectiveness is greater than 90%.

Results: On post-treatment day 1, *G. salmonis* counts taken from fish treated with 100 mg hydrogen peroxide/L (P = 0.0265), but not those treated with 50 mg hydrogen peroxide/L (P = 0.0609), were statistically significantly different than counts taken from untreated control fish. On post-treatment day 14, parasite counts from both the 50 mg/L (P = 0.0052) and 100 mg/L (P = 0.0050) treatment groups were statistically significantly different than the counts from the control group. Percent effectiveness is reported in Table 4.

The percent effectiveness for reducing *G. salmonis* count was greater than 90% in both active treatment groups relative to the untreated control group except for the 50 mg/L of hydrogen peroxide/L treatment group which demonstrated 83% effectiveness for reducing *G. salmonis* counted on Day 1 post-treatment, as shown in Table 4.

Table 4: Geometric mean parasite abundance and percent effectiveness at each post-treatment time point based on the estimated means from the generalized linear mixed model.

Hydrogen peroxide dose (mg /L)	Geometric mean abundance (Post-treatment day 1)	Percent effectiveness (Post-treatment day 1)	Geometric mean abundance (Post-treatment day 14)	Percent effectiveness (Post-treatment day 14)
0	8.30	NA	8.20	NA
50	1.41	83%	0.07	99%
100	0.25	97%	0	100%

Samples of *Gyrodactylus* were definitively identified as *G. salmonis* using an appropriate taxonomic key.

Mean dissolved oxygen (across the tanks in each treatment group) ranged from 9.9–10.1 mg/L, mean pH ranged from 7.81–7.88, and mean flow rate ranged from 589-595 mL/min across the three treatment groups. Mean water temperature was 9.0 °C.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentrations of 50 and 100 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: Results from this study demonstrate that hydrogen peroxide administered as a static bath at concentrations of 50 mg/L for 60 minutes per day, or 100 mg/L for 30 minutes per day, on three alternate days was effective for the treatment and control of *G. salmonis* in freshwater-reared lake trout.

5. Field Effectiveness Study

Title: The Efficacy of 35% PEROX-AID® (Hydrogen Peroxide) to Control Infestations of *Gyrodactylus salmonis* in freshwater-reared rainbow trout *Oncorhynchus mykiss*. H2O2-10-EFF-GYRO.1-01.

Study Dates: October 15, 2010, to October 27, 2010

Study Location: Ennis, Montana, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of hydrogen peroxide administered in a static water bath at a concentration of 50 mg/L for 30 minutes per day on two alternate days to control infestations of *Gyrodactylus salmonis* in freshwater-reared rainbow trout *Oncorhynchus mykiss*. This study was conducted in accordance with Good Clinical Practice.

Study Animals: 150 adult rainbow trout; mean length: 45.6 cm, mean weight: 1.3 kg.

Experimental Design: At the beginning of the study, 20 fish were randomly assigned to each of the six test tanks. Three tanks were assigned to receive hydrogen peroxide treatment and three were untreated control tanks. Each tank was considered to be an experimental unit.

Drug Administration: Fish in treated tanks were treated with hydrogen peroxide as a static bath at a dosage of 50 mg/L for 30 minutes per day on two alternate days. Fish in control tanks received a sham treatment of hatchery water as a static bath for 30 minutes per day on two alternate days.

Measurements and Observations: Before treatment, 30 fish from the reference population were indiscriminately collected for fish health evaluations. Fish were examined externally and internally for abnormalities. Skin scrapes were taken from all fish sampled and *G. salmonis* were counted and recorded. The primary response variable was *G. salmonis* abundance (count) on post-treatment day 7. Abundance (count) was also assessed on post-treatment day 2. On post-treatment day 2, skin scrapes were taken from 10 fish indiscriminately collected from each tank, and *G. salmonis* were counted and recorded. Fish were marked and returned to the tank. On post-treatment day 7, skin scrapes were taken from the 10 fish in each tank that were not already counted, and *G. salmonis* were counted and recorded. Mortality, behavior, appetite, and water quality were monitored. Concentration of hydrogen peroxide was verified from water samples collected from two randomly chosen test tanks on each treatment day.

Statistical Methods: The statistical model included treatment as a fixed effect and tank-within-treatment as a random effect. *G. salmonis* counts were logarithmically transformed for analysis. The hypothesis of equality of means was tested at two-sided $\alpha = 0.05$. Percent effectiveness in the reduction of

mean *G. salmonis* count in treated fish relative to control fish was calculated using geometric means.

percent effectiveness =
100 × (1 – geometric mean treated/geometric mean control)%

Results: A statistically significant difference (P = 0.0004) was detected between the mean abundance of *Gyrodactylus* in treated and control tanks on post-treatment days 2 and 7. Hydrogen peroxide was greater than 90% effective with respect to the number of *Gyrodactylus* counted/fish (treated tanks relative to control tanks) at the end of the study.

Table 5: Geometric mean abundance of *G. salmonis* and percent effectiveness (treated tanks relative to control tanks) on post-treatment days 2 and 7.

Hydrogen peroxide dose (mg /L)	Geometric mean abundance (Post-treatment day 2)	Percent effectiveness (Post-treatment day 2)	Geometric mean abundance (Post-treatment day 7)	Percent effectiveness (Post-treatment day 7)
0	15.69	NA	13.93	NA
50	0.10	99.4%	0.05	99.6%

Samples of *Gyrodactylus* spp. were definitively identified as *G. salmonis* using an appropriate taxonomic key.

Mean hardness, alkalinity, and pH were 254 mg/L $CaCO_3$, 146 mg/L $CaCO_3$, and 8.0, respectively. Mean water temperature was 12.0°C, and mean dissolved oxygen concentration was 7.2 mg/L.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentration of 50 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: Results from this study demonstrate that hydrogen peroxide administered as a static bath at a concentration of 50 mg/L for 30 minutes per day on two alternate days was effective for the treatment and control of *Gyrodactylus salmonis* in freshwater-reared rainbow trout.

6. Field Effectiveness Study

Title: Confirmation of the efficacy of 35% PEROX-AID® to reduce *Gyrodactylus salmonis* infestation density on coaster brook trout *Salvelinus fontinalis*. Study Number AEH-08-PEROXAID-02.

Study Dates: December 3, 2008, to December 22, 2008

Study Location: Iron River, Wisconsin, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of hydrogen peroxide to reduce *Gyrodactylus salmonis* infestation density on coaster brook trout, Salvelinus *fontinalis* with or without salt pretreatment. This study was conducted in accordance with Good Clinical Practice.

Study Animals: 328 female and 314 male adult, post-spawn, coaster brook trout were selected for the study. Male fish weighed an average of 319.9 grams and female fish weighed an average of 213.0 grams.

Experimental Design: The fish were held in three raceways, each containing five compartments. The compartments shared water, which flowed from one end of the raceway to the other. Forty (40) fish, 20 males and 20 females, were randomly assigned to each of the 15 test compartments. There were five different treatment groups with three replicates of each group: 1) a nontreated control group; 2) a group which received a 30 parts per thousand (ppt) salt (NaCl) static immersion bath for 15 minutes, followed by a 15-minute spring water flush, followed by a 15-minute static immersion bath exposure at 150 mg hydrogen peroxide/L; 3) a group which received a 15-minute static immersion bath exposure at 150 mg hydrogen peroxide /L; 4) a group which received a 30 ppt salt static immersion bath exposure for 15 minutes, followed by a 15-minute spring water flush, followed by a 30-minute static bath exposure at 100 mg hydrogen peroxide /L; or 5) a group which received a 30-minute static immersion bath exposure at 100 mg hydrogen peroxide/L. Each test compartment was considered to be one experimental unit.

Table 6. The following table depicts the drug concentrations and treatment group location in the raceways. Each entry represents a single compartment. Water flowed in the direction of top to bottom in this table.

Raceway 1	**Raceway 2**	**Raceway 3**
150 mg H_2O_2/L	100 mg H_2O_2/L + NaCl	150 mg H_2O_2/L + NaCl
100 mg H_2O_2/L	150 mg H_2O_2/L	100 mg H_2O_2/L + NaCl
150 mg H_2O_2/L + NaCl	100 mg H_2O_2/L	Control (0 mg H_2O_2/L)
Control (0 mg H_2O_2/L)	Control (0 mg H_2O_2/L)	100 mg H_2O_2/L
100 mg H_2O_2/L + NaCl	150 mg H_2O_2/L + NaCl	150 mg H_2O_2/L

Drug Administration: Fifteen or 30-minute hydrogen peroxide exposures were administered once daily as a static immersion bath exposure on alternate days for a total of three exposures. The fish were transferred from the test compartments to separate tanks for treatment administration. The first exposure was initiated within 24 hours of fish assignment to the test compartment. The control group received no salt or hydrogen peroxide treatment.

Measurements and Observations: Before transfer to the test compartments, parasite samples (via skin scrape) were collected from 60 fish (30 males and 30 females) from the reference population to determine parasite abundance. Skin scrapes were collected from 20 fish per test compartment (10 males and 10 females) on post-treatment days 1 and day 14, and preserved in formalin. Study fish were sedated with tricaine methanesulfonate and skin scrapes were collected from the area just below the dorsal fin. Fish sampled on post-treatment day 1 were fin-clipped to preclude sampling on post-treatment day 14. Dissolved oxygen, temperature, and pH were monitored in each raceway. Dissolved oxygen and temperature were measured in treatment tanks during each treatment. Salt concentrations were measured in treatment tanks during each salt pre-treatment. To verify hydrogen peroxide concentrations in the treatment tanks, water samples were taken from the treatment water 5 minutes before ending the treatment

Statistical Methods: The post-treatment day 14 parasite counts were analyzed using a generalized linear mixed model with a log link and an overdispersion parameter. The effects of treatment, sex, and treatment by sex (fixed) and raceway (random) were included in the model. A covariate based on position in the raceway was also included to account for the flow of parasites within raceways from upstream to downstream compartments. Treatment and treatment by sex were tested at significance level $\alpha = 0.05$. Pairwise comparisons were made between the hydrogen peroxide-treated group means and the control group mean as appropriate at two-sided $\alpha = 0.05$.

The geometric means for the treatment and control group were estimated using back-transformed least squares mean estimates, and percent effectiveness was computed using the following formula:

percent effectiveness =
$100 \times (1 - \text{geometric mean treated/geometric mean control})\%$

Results: On post-treatment day 14, the mean parasite counts from each hydrogen peroxide-treated group were statistically significantly different than the counts from the control group. The percent effectiveness was greater than 90% in all treated groups (Table 7).

Table 7: Geometric mean abundance and percent effectiveness for an effectiveness study in coaster brook trout treated on three alternate days followed by a 14-day post-treatment period.

Hydrogen peroxide dose (mg /L)	Geometric mean abundance	P-values	Percent effectiveness
0	43.44	n/a	n/a
100	0.50	<0.0001	98.8
100 + 30 ppt NaCl	0.34	<0.0001	99.2
150	0.76	<0.0001	98.2
150 + 30 ppt NaCl	0.31	<0.0001	99.3

Across the three raceways, the water temperature ranged from 4.6 to 5.9 °C; the dissolved oxygen ranged from 10.0 to 11.3 mg/L; and pH ranged from 7.16 to 8.13. In the treatment compartments, the temperature ranged from 5.4 to 6.9 °C; the dissolved oxygen ranged from 7.9 to 11.2 mg/L; and if applicable, salinity ranged from 20 to 30 ppt.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentrations of 100 and 150 mg/L.

Adverse Reactions: No adverse reactions related to test article administration were reported in this study. One mortality occurred during the study on post-treatment day 9 in a compartment treated with 100 mg hydrogen peroxide/L. A single mortality in a large population, occurring so long after treatment is not likely to be test-article related. The fish was excluded from the analysis.

Conclusions: The results of this study demonstrate the effectiveness of hydrogen peroxide applied at 100 mg/L for a 30 minute bath every other day for three treatments for the control of *Gyrodactylus salmonis* on coaster brook trout (*Salvelinus fontinalis*). Treatments using the higher concentration of 150 mg/L for 15 minutes or including pretreatment with salt did not increase effectiveness and are not proposed for this approval.

7. Supportive Field Effectiveness Study

Title: Field effectiveness of 35% PEROX-AID® to reduce *Gyrodactylus* spp. infestation density on coaster brook trout *Salvelinus fontinalis*. Study Number CAP-00-PARASITES-01.

Study Dates: February 4, 2009, to March 6, 2009

Study Location: Marquette, Wisconsin, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of hydrogen peroxide to reduce *Gyrodactylus salmonis* infestation density on coaster brook trout, *Salvelinus*

fontinalis. This study was not conducted in strict adherence with the standards of Good Clinical Practice because it did not include masking or randomization; however, the study was of high enough quality to provide supportive evidence for the effectiveness of 35% PEROX-AID®.

Study Animals: 30,631 juvenile coaster brook trout (Assinica strain); mean length: 14 cm, mean weight: 25.3 g.

Experimental Design: Fish were contained within one raceway, which was divided into two compartments. During treatment, a waterproof barrier was inserted to separate the compartments. The untreated control group was in the upstream half of the raceway and the treated group was in the downstream half of the raceway.

Drug Administration: Hydrogen peroxide was administered once daily on three alternate days as a 30-minute static immersion bath at 100 mg hydrogen peroxide/L.

Measurements and Observations: Mean parasite abundance (primary variable) was measured by counting individual *Gyrodactylus salmonis* found in skin scrapes collected from 20 fish from each treatment group, prior to treatment, and at day 13 post-treatment. Mortality was also monitored beginning 13 days prior to treatment and continuing for 13 days after treatment.

Statistical Methods: The geometric mean for each treatment group on day 13 post-treatment was computed.

Results: The geometric mean number of parasites per sample from the treated fish decreased from 31.6 in the pre-treatment evaluation to 2.5 parasites per sample on Day 13 post-treatment. The geometric mean of the control fish increased from 18.5 in the pre-treatment evaluation to 20.6 parasites per sample on Day 13 post-treatment.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: This study supports the effectiveness of hydrogen peroxide under field conditions when applied at 100 mg/L for a 30 minute bath every other day for three treatments for the control of *Gyrodactylus salmonis* on juvenile brook trout.

8. Field Effectiveness Study

Title: The Efficacy of Hydrogen Peroxide to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*. Study number H202-07-EFF.1-02.

Study Dates: August 11, 2008, to August 30, 2008

Study Location: Webster, Florida, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of hydrogen peroxide as a static bath at a concentration of 50 mg/L for 60 minutes per day on three alternate days to control mortality in fingerling largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*. This study was conducted in general accordance with Good Clinical Practice.

Study Animals: 2,045 fingerling largemouth bass, approximately nine months old were selected for the study. Mean weight of the bass was 17.7 g, mean length was 12.5 cm.

Experimental Design: Test fish were randomly transferred from the reference population into each of eight test tanks. There were four hydrogen peroxide treated tanks and four non-treated (control) tanks, with approximately 250 fish per tank. Each tank was considered to be an experimental unit.

Drug Administration: Fish in treated tanks were treated with hydrogen peroxide as a static bath at a concentration of 50 mg/L for 60 minutes per day on three alternate days. Fish in control tanks received a sham treatment of hatchery water as a static bath for 60 minutes per day on three alternate days. At the end of the 60-minute treatment period, water flow to each tank was resumed.

Measurements and Observations: Cumulative mortality was the primary variable measured. Before treatment, moribund fish from the reference population showing signs of external columnaris disease were sampled for fish health evaluation. Fish were examined externally and internally for abnormalities, and skin scrapes from external lesions were examined microscopically. Observation of gross lesions and microscopic examination were used to diagnose columnaris. Mortality was observed twice daily during the 5-day treatment period and the 14-day post-treatment period. Concentration of hydrogen peroxide was verified from water samples collected from two randomly chosen test tanks (one treated and one control) on each treatment day. Water temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity, and pH were measured two times during the study.

Statistical Methods: A generalized linear model was used to test for a group effect of the test article on cumulative mortality. The ratios dead/total were analyzed at two-sided $\alpha = 0.05$. The mean cumulative mortality of fish in treated and control tanks was analyzed using SAS PROG GLIMMIX analysis.

Results: A statistically significant difference was detected between treated and control tanks at the end of the study (post-treatment day 14; $P = 0.0075$). Mortality results are summarized in Table 8.

Table 8: Mortality results for Trial 3 challenge study in largemouth bass with a 3 alternate day treatment period and 14-day post-treatment period.

Hydrogen Peroxide Dose (mg /L)	Percent Cumulative Mortality
0	74.7 (748/1002)
50	48.8 (509/1043)

Mean hardness, alkalinity, and pH were 320 mg/L $CaCO_3$, 350 mg/L $CaC0_3$, and 7.6, respectively. Mean water temperature was 24.8 °C, and mean dissolved oxygen concentration was 13.9 mg/L.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentration of 50 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: The results from this study demonstrate the effectiveness of hydrogen peroxide administered at 50 mg/L for 60 minutes per day on three alternate days to control mortality in largemouth bass *Micropterus salmoides* due to external columnaris disease associated with *Flavobacterium columnare*.

9. Field Effectiveness Study

Title: The Efficacy of Hydrogen Peroxide to Control Mortality in Bluegill *Lepomis macrochirus* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*. Study Number H2O2-07-EFF.1-05.

Study Dates: August 31, 2008, to September 19, 2008

Study Location: Webster, Florida, U.S.A.

Study Design:

Objective: To evaluate the effectiveness of hydrogen peroxide as a static bath at a concentration of 50 mg/L for 60 minutes per day on three alternate days to control mortality in bluegill due to external columnaris disease associated with *Flavobacterium columnare*. This study was conducted in general accordance with Good Clinical Practice.

Study Animals: 1268 bluegill *Lepomis macrochirus.* Mean body weight 16.5 g, mean length 9.7 cm.

Experimental Design: Test fish were randomly transferred from the reference population into each of twelve test tanks. There were six hydrogen peroxide treated tanks and six non-treated (control) tanks, with approximately 105 fish per tank. Each tank was considered to be an experimental unit.

Drug Administration: Fish in treated tanks were treated with hydrogen peroxide as a static bath at a concentration of 50 mg/L for 60 minutes per day on three alternate days. Fish in control tanks received a sham treatment of hatchery water as a static bath for 60 minutes per day on three alternate days. At the end of the 60-minute treatment period, water flow to each tank was resumed.

Measurements and Observations: Cumulative mortality was the primary variable measured. Before treatment, moribund fish from the reference population showing signs of external columnaris disease were sampled for fish health evaluation. Fish were examined externally and internally for abnormalities, and skin scrapes from external lesions were examined microscopically. Observation of gross lesions and microscopic examination were used to diagnose columnaris. Mortality was observed twice daily during the 5-day treatment period and the 14-day post-treatment period. Concentration of hydrogen peroxide was verified from water samples collected from two randomly chosen test tanks (one treated and one control) on each treatment day. Water temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity, and pH were measured two times during the study.

Statistical Methods: A generalized linear model was used to test for a group effect of the test article on cumulative mortality. The ratios dead/total were analyzed at two-sided $\alpha = 0.05$. The mean cumulative mortality of fish in treated and control tanks was analyzed using SAS PROG GLIMMIX analysis.

Results: A statistically significant difference was detected between treated and control tanks at the end of the study (post-treatment day 14; $P = 0.0051$). Mortality results are summarized in Table 9.

Table 9: Mortality results for an effectiveness study in bluegill treated on three alternate days followed by a 14-day post-treatment period.

Hydrogen Peroxide Dose (mg /L)	Percent Cumulative Mortality
0	19.3 (125/649)
50	10.2 (62/609)

Mean hardness, alkalinity, and pH were 345 mg/L $CaCO_3$, 370 mg/L $CaC0_3$, and 7.6, respectively. Mean water temperature was 24.9 °C, and mean dissolved oxygen concentration was 15.2 mg/L.

Results for the measured hydrogen peroxide concentration showed that the drug administration was appropriate to achieve the target concentration of 50 mg/L.

Adverse Reactions: No adverse reactions were reported in this study.

Conclusions: The results from this study demonstrate the effectiveness of hydrogen peroxide administered at 50 mg/L for 60 minutes per day on three alternate days to control mortality in bluegill *Lepomis macrochirus* due to external columnaris disease associated with *Flavobacterium columnare*.

III. TARGET ANIMAL SAFETY

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-255 dated January 11, 2007, contains a summary of target animal safety studies for freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish at doses up to 75 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath; for freshwater-reared salmonids at doses up to 50 mg/L for 60 minutes, or 100 mg/L for 30 minutes, per day on alternate days for three treatments in a continuous flow water supply or as a static bath; for freshwater-reared warmwater finfish fry at doses up to 50 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath; and for freshwater-reared warmwater finfish fingerling and adult fish at doses up to 50-75 mg/L for 60 minutes per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

IV. HUMAN FOOD SAFETY

CVM did not require additional information for human food safety. The FOI Summary for the original approval of NADA 141-255 dated January 11, 2007, contains a summary of all information used to assess human food safety. Neither an acceptable daily intake (ADI), tolerance, withdrawal time, nor regulatory methods are assigned.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to 35% PEROX-AID®:

USER SAFETY WARNINGS

Not for use in humans. Keep out of reach of children.

INHALATION (Breathing):

- Avoid breathing vapor or mist; causes irritation of the nose, throat and lungs; exposure may be fatal.

INGESTION (Swallowing):

- Do not swallow; this product is harmful if swallowed; large exposures may be fatal; can burn mouth, throat, and stomach.

EYE CONTACT:

- Do not get in eyes; causes eye burns and possible blindness; effects may be delayed.

SKIN CONTACT:

- Avoid contact with skin; causes skin irritation or burns.

HUMAN PRECAUTIONS

- Wear chemical safety goggles.

- Wear neoprene, butyl, or vinyl gloves.
- Keep out of reach of children.
- Use only in adequate ventilation.
- Keep containers tightly closed when not in use.
- Wear suitable protective clothing.

EMERGENCY FIRST AID

- In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Call a physician.
- Remove and wash contaminated clothing and shoes promptly and thoroughly.
- If inhaled, move to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.
- If swallowed, do not induce vomiting. Give large quantities of water. Never give anything by mouth to an unconscious person. Call a physician.
- Bring the package insert with you to the health care professional.

NOTE TO PHYSICIAN: If swallowed, large quantities of oxygen may be released quickly. The distension of the stomach or esophagus may be injurious. Insertion of a gastric tube may be advisable.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that 35% PEROX-AID®, when used according to the label, is safe and effective for the control of mortality in freshwater-reared coldwater finfish, fingerling and adult freshwater-reared coolwater finfish, and fingerling and adult freshwater-reared warmwater finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae; for the treatment and control of *Gyrodactylus* spp. in freshwater-reared salmonids; and for the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*. Additionally, data demonstrate that residues in food products derived from species treated with 35% PEROX-AID® will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

B. Exclusivity

This supplemental approval for 35% PEROX-AID® qualifies for SEVEN years of exclusive marketing rights beginning as of the date of our approval letter. This drug qualifies for exclusive marketing rights under section 573(c) of the FD&C Act because it is a designated new animal drug under section 573(a) of the FD&C Act. Except as provided in section 573(c)(2) of the FD&C Act, we may not approve or conditionally approve another application submitted for such new animal drug with the same intended use as 35% PEROX-AID®. 35% PEROX-AID®,

as approved in this supplement, does not qualify for marketing exclusivity under section 512(c)(2)(F) of the FD&C Act.

Note that the SEVEN years of exclusivity granted for the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare* applies for all freshwater-reared warmwater finfish except channel catfish. Approval for this indication for channel catfish was granted in the original approval of NADA 141-255 dated January 11, 2007, and seven years exclusive marketing rights for that indication began on that date.

C. Supplemental Applications

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.