Date of Approval: JAN 11 2007

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-255

35% PEROX-AID

Hydrogen peroxide
Liquid solution

"For the control of mortality in freshwater-reared finfish eggs due to saprolegniasis,

For the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*,

And

For the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *Flavobacterium columnare (Flexibacter columnaris)*."

Sponsored by:

Eka Chemicals, Inc.

2007-141-255

FOIS 1

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number: NADA 141-255

B. Sponsor: Eka Chemicals, Inc.
1775 West Oak Commons Ct.
Marietta, GA 30062-2254

Drug Labeler Code: 061088

C. Proprietary Name: 35% PEROX-AID

D. Established Name: Hydrogen peroxide

E. Pharmacological Category: External disinfectant

F. Dosage Form: Liquid solution

G. Amount of Active Ingredient: 35% w/w (weight in water)

H. How Supplied: 5-gallon and 55-gallon containers

I. How Dispensed: Over-the-counter (OTC)

J. Dosages: Freshwater-reared finfish eggs: 500 to 1000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all coldwater and coolwater species of freshwater-reared finfish eggs or 750 to 1000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all warmwater species of freshwater-reared finfish eggs.

Freshwater-reared finfish:

Freshwater-reared salmonids: 100 mg/L for 30 minutes or 50 to 100 mg/L for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

Coolwater species of freshwater-reared **finfish** (except northern pike & paddlefish) and channel catfish*: 50 to 75 mg/L for 60 minutes once per

day on alternate days for three treatments in a continuous flow water supply or as a static bath. Coolwater species of freshwater-reared **finfish fry** (except northern pike, pallid sturgeon & paddlefish) and channel catfish fry*: 50 mg/L for 60 minutes once per day on alternate days for three treatments in continuous flow water supply or as a static bath.

*Initial bioassay on a small number is recommended before treating the entire group. Use with caution on walleye.

K. Route of Administration: Immersion

L. Species/Classes: Freshwater-reared finfish eggs; and freshwater-reared salmonids, coolwater finfish and channel catfish

M. Indications: For the control of mortality in freshwater-reared finfish eggs due to saprolegniasis, for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*, and for the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *Flavobacterium columnare (Flexibacter columnaris)*

II. EFFECTIVENESS:

The data summarized in this section are publicly available and contained in Public Master File 005639 and Investigational New Animal Drug File 010023 which were compiled by the U.S. Geological Survey, Upper Midwest Environmental Sciences Center.

A. Dosage Characterization:

The primary effect of hydrogen peroxide results from localized action at the topical site of administration. The concentration of the active drug at the topical site is a function of the administered concentration, exposure period, and water conditions. These three conditions and the sensitivity of the pathogen to the drug are considered the primary determinants of effectiveness. The dosage characterization studies included one non-treated group and different concentrations of hydrogen peroxide in water. The effectiveness of hydrogen peroxide at specific concentrations and exposure times was tested for the control of mortality associated with bacterial gill disease on freshwater-reared finfish and for the control of egg mortality associated with saprolegniasis on all cold- and coolwater species of freshwater-reared finfish.

B. Substantial Evidence for the Control of Mortality in Freshwater-Reared Finfish Eggs Due to Saprolegniasis:

1. Clinical Field Trial- Study No. CAP-00-FUNGUS

Title: Efficacy of Hydrogen Peroxide to Control Mortality Associated with Saprolegniasis in Channel Catfish *(Ictalurus punctatus)* Eggs

Investigator: Tommy Crawford

Study Location: Lost Valley Fish Hatchery
Warsaw, MO

General Design of the Study:

a. Purpose: To evaluate the effectiveness of daily 15-minute treatments with 500 and 750 mg/L hydrogen peroxide over 6 days to control egg mortality associated with saprolegniasis as measured by the probability of egg hatch.

b. Animals: Channel catfish eggs, contained in their natural gelatin matrix and naturally infected with *Saprolegnia parasitica*, were used.

c. Test Article: Hydrogen peroxide (35%) was used in the study.

d. Study Design: Egg masses of 0.79 kg (equivalent to approximately 17,500 non-eyed eggs), in the natural gelatin matrix, were randomly assigned among nine

McDonald egg jars to three treatment concentrations: 0 (control), 500, and 750 mg/L. Eggs were treated for 15 minutes daily for 6 days until eggs hatched. Egg samples were collected for disease confirmation; subculture produced zoospores that were identified as *Saprolegnia parasitica.*

e. Variables Measured: Mean percent hatch was the primary variable used to evaluate effectiveness of the treatment. Temperature, dissolved oxygen, and pH were monitored daily. During each treatment, a water sample was collected from each egg jar and analyzed for hydrogen peroxide concentration using a permanganate titration method.

f. Results: Table 1 summarizes the mean percent hatch of channel catfish eggs after hydrogen peroxide treatment.

Table 1. Mean percent hatch of channel catfish eggs after treatment with hydrogen peroxide for 15 minutes daily for 6 days.

Treatment mg/L	**Hatch (%)**
0	44
500	54
750	69

Mean temperature of the hatchery water was 27.8 °C. Total hardness was 150 mg/L; alkalinity was 130 mg/L; and pH ranged from 8.09 to 8.20. Mean dissolved oxygen readings ranged from 7.4 to 10.5 mg/L. Mean hydrogen peroxide concentrations were within +/- 20% of the target concentrations.

Mean hatch rate for eggs treated with 750 mg/L hydrogen peroxide was approximately 1.5 times greater than the mean hatch of the control.

g. Conclusions: This study demonstrates the effectiveness of hydrogen peroxide at 750 mg/L for 15 minutes daily until hatch to control mortality in channel catfish eggs due to saprolegniasis. Hydrogen peroxide is clinically effective in controlling mortality due to *Saprolegnia parasitica* as supported by a numerical increase in hatch rate in channel catfish eggs compared with controls.

2. Study TOX-89-00048

Title: Efficacy of Hydrogen Peroxide Treatments to Control and Prevent Saprolegniasis Infections on Salmonid Eggs

Study Director: Jeffrey J. Rach

Investigators: Jeffrey J. Rach, Theresa M. Schreier, George E. Howe

Study Location: US Geological Survey
Upper Mississippi Science Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the effectiveness of hydrogen peroxide administered at concentrations of 113, 283, 566, and 1132 mg/L (100, 200, 500, and 1000 µL/L) for 15 minutes every other day until hatch to control saprolegniasis on rainbow trout eggs.

b. Animals: Each treatment consisted of three replicates of 500 non-eyed fertilized rainbow trout eggs, 36-hours old, in each of the two trials. Each trial used eggs from a different lot (different parents and different time of year).

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Two trials were conducted. In Trial 1, hydrogen peroxide was administered at 0, 113, 283 or 566 mg/L. In Trial 2, hydrogen peroxide was administered at 0 or 1132 mg/L. Eggs were exposed for 15 minutes every other day until they hatched. Studies in both trials were conducted on groups with induced *Saprolegnia parasitica* infections (10% of eggs visibly infected) at the start of treatment. Eggs were placed in tanks with constant flow for 53 days.

   This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables Measured: Mortality and fungal infection rates were recorded before the first and the last treatment. Fry hatch was assessed after all viable eggs completed the process. Percent hatch was corrected for initial mortality using the formula [(Number of eggs hatched)/(500 eggs-initial mortalities)] x 100. Samples of water were collected for hydrogen peroxide concentration analysis by permanganate titrimetric analysis. Water pH, dissolved oxygen, and temperature were recorded daily.

f. Results: Mean infection rates and mean hatch of rainbow trout eggs infected with *Saprolegnia parasitica* and treated with hydrogen peroxide are summarized in Tables 2 and 3 (Trials 1 and 2).

Table 2. Trial 1: Infection rates of rainbow trout eggs infected with *Saprolegnia parasitica* and mean hatch rates after treatment with hydrogen peroxide for 15 minutes every other day.

Hydrogen peroxide (mg/L)	**Mean Initial Infection (%)**	**Mean Final Infection (%)**	**Mean Hatch (%)**
0	150/1500 (10%)	811/1500 (54%)	727/1453 (50%)
113	151/1500 (10%)	740/1500 (49%)	817/1455 (56%)
283	150/1500 (10%)	577/1500 (38%)	944/1460 (65%)
566	150/1500 (10%)	385/1500 (26%)	1166/1458 (80%)

Table 3. Trial 2: Infection rates of rainbow trout eggs infected with *Saprolegnia parasitica* and mean hatch rates after treatment with hydrogen peroxide for 15 minutes every other day.

Hydrogen peroxide (mg/L)	**Mean Initial Infection (%)**	**Mean Final Infection (%)**	**Mean Hatch (%)**
0	150/1500 (10%)	1019/1500 (68%)	385/1406 (27%)
1132	150/1500 (10%)	435/1500 (29%)	964/1411 (68%)

Measured hydrogen peroxide concentrations ranged from 102.4 to 111.9% of expected values. Water temperature ranged from 11.4 to 14.5 °C; dissolved oxygen ranged from 9.6 to 13.5 mg/L; and pH ranged from 7.66 to 8.46.

g. Substantiating literature: Marking LL, JJ Rach, and TM Schreier. Evaluation of antifungal agents for fish culture. *The Progressive Fish Culturist*. 56: 225-240, 1998. This published article provides additional evidence for the effectiveness of hydrogen peroxide to control mortality of salmonid eggs infected by *Saprolegnia spp.* and supports the conclusion drawn from Study TOX-89-00048.

Conclusions: This study, together with substantiating literature, demonstrates the effectiveness of hydrogen peroxide at 566 and 1132 mg/L for 15 minutes every other day until hatch to control mortality in salmonid eggs due to saprolegniasis. Continuous flow treatments with hydrogen peroxide at 566 mg/L (Trial 1) and 1132 mg/L (Trial 2) for 15 minutes every other day were clinically effective in the control of mortality of rainbow trout eggs associated with *Saprolegnia parasitica* infection.

3. Clinical Field Trial – Study TOX-94-0048-2

Title: Effectiveness of Hydrogen Peroxide to Control Mortality Associated with Saprolegniasis on Walleye *(Stizostedion vitreum)* and White Sucker *(Catostomus commersonii)* Eggs

Study Director: Jeffrey J. Rach

Study Location: US Geological Survey
Upper Midwest Experimental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To determine the effectiveness of hydrogen peroxide to control mortality associated with fungal infections associated with saprolegniasis on walleye and white sucker eggs as measured by the probability of egg hatch.

b. Animals: Non-eyed eggs of walleye and white sucker were used.

c. Test Article: Hydrogen peroxide (35%) was used in the study.

d. Study Design: For each species, 30 mL volumes of non-eyed eggs were assigned to four test groups in egg hatching systems. Approximately 2931 walleye eggs and 639 white sucker eggs (30 mL) were placed in each hatch container. Water flow was monitored and adjusted daily to maintain a flow of 240 +/- 25 mL/minute. Hydrogen peroxide was administered at 0, 283, 565, or 1130 mg/L for 15-minute exposures every other day until eggs hatched. Walleye eggs were treated four times, and white sucker eggs received five treatments. Eggs were incubated at 12 +/- 2 °C.

e. Variables Measured: The primary variable considered was the percent hatched fry after the treatment. Concentrations of hydrogen peroxide were analyzed for each treatment using a permanganate titrimetric method. Temperature, dissolved oxygen, and pH were measured in all containers during the study.

f. Statistical Analysis: Cumulative mortality of non-eyed walleye and white sucker eggs was analyzed using a general linear model ANOVA with binomial distribution and logit link, using the SAS procedure GENMOD at the 0.05 level of significance.

g. Results: Fungal infections (visible fungus) spread to most of the untreated control eggs whereas the treated eggs had reduced or no visible fungus on the eggs. Effectiveness of hydrogen peroxide treatment on walleye and white sucker eggs is summarized in Table 4. Mean hydrogen peroxide concentrations in the water during the study were within 15% of expected concentrations.

Table 4. Mean percent hatch of walleye and white sucker non-eyed eggs after the treatment with hydrogen peroxide for 15 minutes every other day.

Eggs **Fish Species**	**Hydrogen Peroxide (mg/L)** Mean Hatch (%)			
	0	**283**	**565**	**1130**
Walleye (% hatch)	11	55*	49*	65*
White Sucker (% hatch)	9	23*	47*	69*

*Percent hatch significantly different from control ($p \leq 0.05$).

h. Conclusions: This study supports the effectiveness of hydrogen peroxide in a flow-through system at 283, 565, and 1130 mg/L for 15 minutes every other day until hatch to control mortality associated in walleye and white sucker eggs due to saprolegniasis. Effectiveness in this study is evidenced by increased hatching success.

4. Clinical Field Trial – Study TOX-94-0048-2

Title: Efficacy of Hydrogen Peroxide to Control Mortality Associated with Saprolegniasis Caused by *Saprolegnia parasitica* in Walleye Eggs (*Stizostedion vitreum)*

Investigator: Lynn A. Lee

Study Location: US Geological Survey
Upper Midwest Experimental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the effectiveness of 500 and 750 mg/L hydrogen peroxide administered as a flow-through treatment for 15 minutes on 12 consecutive days to control mortalities from saprolegniasis caused by *Saprolegnia parasitica* on walleye eggs.

b. Animals: Using von Bayer egg counts, an estimated 2.398 million eggs (14.36 kg) were used in this trial. Fertilized eggs were mixed with a solution of Fuller's earth and water.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: There were three treatment groups: 0 (control), 500 and 750 mg/L hydrogen peroxide. There were three jars (replicates) for each of the two treatment groups, and two jars (replicates) for the untreated controls. A partially randomized design was used to assign treatments to the McDonald egg jars. Eggs were randomly distributed to jars; approximately 326,000 eggs were placed in each jar. Hydrogen peroxide was administered as a continuous flow exposure for 15 minutes each day for 12 treatments. Eggs were assumed to have fungal zoospores upon study initiation; during the study a composite sample of eggs with visible fungal growth was collected and submitted to a laboratory for definitive identification.

e. Variables Measured: Survival was estimated by dividing the total number of fry that hatched from each jar by either the initial number of non-eyed eggs or estimated number of eyed eggs from samples removed on Day 8. Water hardness and alkalinity were measured twice during the experiment. Water temperature, dissolved oxygen, and pH were measured in each egg jar during treatment. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Hatching success was analyzed using a generalized linear model employing a binomial distribution and logit link at the 0.05 level of significance.

g. Results: The fungus in the composite egg sample was identified as *Saprolegnia parasitica*.

Effectiveness of hydrogen peroxide treatment on eggs is summarized in Table 5.

Table 5. Mean percent hatch of walleye eggs with saprolegniasis after the treatment with hydrogen peroxide for 15 minutes every day for 12 treatments.

	Hydrogen Peroxide mg/L Mean Hatch (%)		
	0	**451**	**695**
Non-eyed Eggs	48.6	53.1*	49.6
Eyed Eggs	66.0	78.2*	70.0

*Hatch rate differs significantly from controls ($p \leq 0.05$).

Verified mean hydrogen peroxide concentrations were 451 mg/L for the 500 mg/L jars and 695 mg/L for the 750 mg/L jars.

h. Conclusions: This study supports the effectiveness of hydrogen peroxide at 451 mg/L in a flow-through system for 15 minutes daily to control mortality in walleye eggs due to saprolegniasis.

C. Substantial Evidence for the Control of Mortality in Freshwater-Reared Salmonids Due to Bacterial Gill Disease Associated with *Flavobacterium branchiophilum*:

1. Clinical Field Trial - Study No. CAP-97-0048-09

Title: Pivotal Studies to Evaluate the Efficacy of Hydrogen Peroxide to Control Mortalities Associated with External Flavobacter Infections on Cultured Fish at Selected Fish Hatcheries

Study Directors: Jeffrey J. Rach

Investigator: Mark P. Gaikowski

Study Location: US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the effectiveness of hydrogen peroxide as a static bath at concentrations of 0, 57, 113, and 226 mg/L for 30 minutes (rainbow trout, *Oncorhynchus mykiss*) and 60 minutes (brown trout, *Salmo trutta*, and Chinook salmon, *Oncorhynchus tschawytscha*) every other day for three treatments to control mortality in fish with bacterial gill disease.

b. Animals: Fingerling brown trout (9 g), Chinook salmon (3.5 g), and rainbow trout (10 g) were used in the study.

c. Test article: Hydrogen peroxide (35% w/w) was used on the treated fish.

d. Study Design: All fish were cultured in outdoor raceways and were naturally infected with bacterial gill disease. After microscopic diagnosis of the disease, fish were removed from the source raceway and hand-counted according to random assignment into each test tank (brown trout, $n = 20$; Chinook salmon, $n = 44$; rainbow trout, $n = 28$). Four treatment groups were used for each species: 0 (control), 57, 113, and 226 mg/L. The study was conducted using fish held in three tanks of 20 and 40 L. Water flow was 187 mL/min for Chinook salmon, 1.5 L/min for brown trout, and 1.9 L/min for rainbow trout. Fish were exposed to a static hydrogen peroxide bath once every other day for 3 treatments. Each treatment was replicated three times. Brown trout were observed for mortality for 12 days after the last treatment, while the post-treatment observation period was 14 days for rainbow trout and Chinook salmon.

A clinical diagnosis of bacterial gill disease was made if fish were lethargic, rode high in the water, oriented to water flow, or rejected food. Bacterial gill disease is a superficial infection of the gill epithelia by filamentous bacteria, *Flavobacterium branchiophilum*, although other opportunistic Gram-negative bacteria have been associated with the disease. Diagnosis was confirmed by microscopic examination of gill swabs and verification of the presence of filamentous bacteria.

This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables measured: Cumulative mortality was recorded daily during the 5-day treatment period and the 12- or 14-day observation period after the last treatment. Dissolved oxygen, temperature, and pH of the water were recorded daily in each test tank. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Cumulative mortality was analyzed using a general linear model analysis of variance (ANOVA) with binomial distribution and logit link using the GENMOD procedure in SAS at the 0.05 level of significance.

g. Results: Cumulative mortality through the end of the post-treatment observation period is summarized in Table 6.

Table 6: Cumulative mean mortality (%) at 12 (a) or 14 (b) days after 30- or 60-minute treatments every other day for three treatments.

H_2O_2 Concentration (mg/L)	**Brown Trout (a) (60-min. bath)**	**Chinook Salmon (b) (60-min. bath)**	**Rainbow Trout (b) (30-min. bath)**
0	11.7^{a}	21.2^{a}	31.0^{a}
57	0.0^{b}	7.6^{a}	25.0^{ab}
113	6.7^{a}	22.0^{a}	14.3^{bc}
226	36.7^{c}	72.0^{b}	7.1^{c}

^{a,b,c} Percentages with different superscripts differ ($p \leq 0.05$)

Hydrogen peroxide concentrations were within +/- 10% of the target concentrations.

h. Conclusions: This study demonstrates the clinical effectiveness of hydrogen peroxide at 57 mg/L for 60 minutes and 113 mg/L for 30 minutes every other day for three treatments to control mortality in freshwater-reared salmonids due to bacterial gill disease. The data in this study show that for brown trout fingerlings, hydrogen peroxide at 57 mg/L administered as a 60-minute static bath

significantly reduces mortality associated with bacterial gill disease as compared to the controls. For rainbow trout fingerlings, hydrogen peroxide at 113 mg/L administered as a 30-minute static bath significantly reduces mortality as compared to the controls. Mortality among brown trout and Chinook salmon in the 226 mg/L groups (1 hour treatment) is significantly higher than among control groups and is consistent with dose-related toxicity.

2. **Supporting Data – Published Literature**

Source: Lumsden JS, VE Ostland, and HW Ferguson. Use of Hydrogen Peroxide to Treat Experimentally Induced Bacterial Gill Disease in Rainbow Trout. *Journal of Aquatic Animal Health.* 10: 230–240, 1998.

Study Location: Fish Pathology Laboratory
University of Guelph
Ontario, Canada

General Design of the Study:

a. Purpose: To determine the efficacy of hydrogen peroxide to control mortality associated with experimentally induced bacterial gill disease in rainbow trout.

b. Animals: Rainbow trout fingerlings weighing 12 to 20 grams were used in the study.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Fingerlings were experimentally infected by challenge in a static tank containing *Flavobacterium branchiophilum* at a concentration of 1×10^5 colony-forming units (CFU) per mL for 1 hour. *F. branchiophilum* challenge concentrations were verified by plate count. Treatments were administered in the same tanks where the fish were infected. Fish were stocked at 100 g/L into experimental tanks. Hydrogen peroxide treatments were administered as a static bath at 0 (control), 25, 75, 100, 125, 175, or 250 mg/L for 60 minutes every other day for two exposures (Experiments 1 and 2) or every day for three exposures (Experiment 3). Treatment was initiated 48 hours post-infection. The disease was diagnosed prior to and after the treatment by identifying the bacteria and associated microscopic lesions.

e. Variables Measured: Cumulative mortality was recorded and analyzed to evaluate effectiveness. Mortality was monitored through the second day after the last death was noted. Hydrogen peroxide concentrations were measured using iodometric titration.

f. Statistical Analysis: Inability to access the underlying data in this publication precluded statistical analysis. Conclusions were drawn based on numerical differences evident in the report.

g. Results: Cumulative mortality in each experiment is summarized in Table 7.

Table 7. Cumulative mortality after treatment with hydrogen peroxide to control bacterial gill disease in rainbow trout.

	Cumulative Mortality (%)		
H_2O_2 (mg/L)	**Exp. #1 (Two treatments EOD)**	**Exp. #2 (Two treatments EOD)**	**Exp. #3 (Three treatments SID)**
0	44.4	61.1	60.0
25	34.1	*	4.2
75	*	*	7.5
100	25.9	21.1	*
125	*	*	13.3
175	*	*	9.2
250	2.2	15.5	24.2

*Not tested

Hydrogen peroxide concentrations were within 10% of the target concentrations.

h. Conclusions: This study supports the effectiveness of hydrogen peroxide at 100 and 250 mg/L for 60 minutes every other day for two treatments to control mortality in rainbow trout due to bacterial gill disease associated with *Flavobacterium branchiophilum*.

D. Substantial Evidence for the Control of Mortality in Freshwater-Reared Coolwater Finfish and Channel Catfish Due to External Columnaris Disease Associated with *Flavobacterium columnare (Flexibacter columnaris)*:

1. Clinical Field Trial – Study No. CAP-00-BACTERIA

Title: Effectiveness of Hydrogen Peroxide to Control Mortality Associated with External Columnaris [*Flavobacterium columnare (Flexibacter columnaris)*] on Channel Catfish *(Ictalurus punctatus)*

Investigators: Alan Johnson, Jeffrey J. Rach

Study Location: Rathbun Fish Hatchery
Moravia, IA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of hydrogen peroxide to control mortality associated with external columnaris on channel catfish fingerlings.

b. Animals: Naturally infected channel catfish fingerlings (2.28 g) from a single production lot were used in the study.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Four treatment groups of fish were included: control (0 mg/L), and treated with hydrogen peroxide at 50, 75, and 100 mg/L. Treatments were administered as a static bath for 60 minutes every other day for three treatments. Each treatment group was replicated three times. After the fish were diagnosed with columnaris, 26 fish were randomly assigned to each of 12 test tanks (312 fish total), and treatments were initiated on the same day without an acclimation period. The last exposure was followed by a 10-day post-treatment observation period. Observation of visual lesions and microscopic examination were used to diagnose the disease.

e. Variables Measured: Mortalities were recorded daily with the initial recording made approximately 24 hours after the first treatment. Dissolved oxygen, temperature and pH were recorded daily in each test tank. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Cumulative survival to post-treatment was analyzed using logistic regression based on the SAS procedure GENMOD. Model fit was based on scaled deviance. Differences between means were determined using least square means at the 0.05 level of significance.

g. Results: Mortality rates after treatment are summarized in Table 8.

Table 8: Percent mortality at the end of the 15-day study (10 days after treating with hydrogen peroxide for 60 minutes every other day for three treatments.)

Treatment Group (mg/L)	% Cumulative Mortality
0	80.8[a]
50	47.4[b]
75	28.2[b]
100	41.0[b]

[a,b] Means with different superscripts differ ($p \leq 0.05$)

Hydrogen peroxide concentrations were within +/- 5% of the target concentrations.

h. Conclusions: This study demonstrates the effectiveness of hydrogen peroxide at or above 50 mg/L for 60 minutes every other day for three treatments to control mortality in channel catfish due to external columnaris disease.

2. Clinical Field Trial- CAP-00-BACTERIA (Rathbun-10-023-Ia-Bacteria-2)

Title: Efficacy of Hydrogen Peroxide to Control Mortality Associated with External Columnaris on Walleye *(Stizostedium vitreum).*

Investigators: Alan Johnson, Jeffrey J. Rach

Study Location: Rathbun Fish Hatchery
Moravia, IA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of hydrogen peroxide to control mortality associated with external columnaris on walleye fingerlings.

b. Animals: A total of 156 walleye fingerlings with mean body weight of 12 g and length ranging from 10 to 13 cm were used in the study.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Fish naturally infected with external columnaris were randomized to treatments with 0 (control), 50, 75, or 100 mg/L hydrogen peroxide administered as a static bath for 60 minutes every other day for three treatments. Each treatment was conducted with three replicate tanks and with 13 fish

allocated to each tank. Before testing began, twelve fish were collected from the reference population for disease diagnosis. Observation of visual lesions and microscopic examination were used to diagnose columnaris. Water flow was suspended during treatment. Aeration was supplied for the duration of the study. Post-treatment mortality data was collected daily for 10 days following the final treatment.

e. Variables Measured: Mortality was recorded and dead fish were removed from the tank daily. Water was analyzed for hardness and alkalinity. Dissolved oxygen, temperature, and pH were recorded daily in each test tank. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Logit transformed cumulative mortality was analyzed using a general linear model ANOVA based on the GENMOD procedure in SAS.

g. Results: Cumulative mortality after the treatment is summarized in Table 9.

Table 9. Mortality rates after hydrogen peroxide treatment of walleye fingerlings infected with external columnaris.

Treatment Group (mg/L)	**% Cumulative Mortality (Days 1-15)**
0	69.2[a]
50	35.9[b]
75	56.4[a]
100	61.5[a]

[a,b] Rates with different superscripts differ ($p \leq 0.05$).

Hydrogen peroxide concentrations were within +/- 12% of the target concentrations. The mean water temperature was 25.3 °C. Water total hardness was 90 mg/L, alkalinity was 75 mg/L, average pH was 7.77, and average dissolved oxygen was 7.9 mg/L.

h. Conclusions: This study demonstrates the effectiveness of hydrogen peroxide at 50 mg/L of water for 60 minutes every other day for three treatments to control mortality in walleye due to external columnaris disease.

3. Supporting Data – Study CAP-97-0048-09

Title: Pivotal Studies to Evaluate the Efficacy of Hydrogen Peroxide to Control Mortalities Associated with External Flavobacter Infections on Cultured Fish at Selected Fish Hatcheries

Study Director: Jeffrey J. Rach

Investigator: Mark P. Gaikowski

Study Location: US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the effectiveness of hydrogen peroxide at 57 and 113 mg/L for 60 minutes in a static bath every other day for three treatments to control mortality associated with external columnaris disease in yellow perch.

b. Animals: Yellow perch weighing approximately 1 g were used in the study.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Prior to treatment, the fish were experiencing a natural outbreak of columnaris with 50% mortality. Observation of visual lesions and microscopic examination were used to diagnose columnaris. Fish were randomly assigned to 20 L tanks and were exposed to hydrogen peroxide in a static bath at concentrations of 0 (control), 57, or 113 mg/L for 60 minutes every other day for three treatments. There were three replicates per treatment concentration. Aeration was maintained during the study and water flow was suspended during treatment period.

   This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables Measured: Mortality was recorded daily in each tank for 14 days, including 9 days after the last treatment. Water temperature, dissolved oxygen, and pH were measured daily. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Results: Cumulative mean mortality after the treatment is summarized in Table 10.

Table 10. Cumulative mortality in yellow perch fingerlings with external columnaris after treatment with 0, 57, or 113 mg/L hydrogen peroxide as a static bath for 60 minutes every other day for three treatments. Mortality was observed for 14 days, beginning with the first day of treatment.

Treatment Group (mg/L)	**% Cumulative Mortality (Days 1-14)**
0	7.5
57	1.1
113	5.2

The water temperature ranged from 12.0 to 13.3 °C, dissolved oxygen from 8.0 to 10.4 mg/L, and pH from 7.64 to 8.11. Hydrogen peroxide concentrations were within +/- 10% of the target concentrations.

g. Conclusions: This study supports the clinical effectiveness of hydrogen peroxide at 57 mg/L for 60 minutes every other day for three treatments to control mortality in yellow perch associated with external columnaris disease.

III. TARGET ANIMAL SAFETY:

Target animal safety was determined by considering studies summarized in this section as well as data provided in effectiveness trials.

The data summarized in this section are publicly available and contained in Public Master File 005639 and Investigational New Animal Drug File 010023 which were compiled by the U.S. Geological Survey, Upper Midwest Environmental Sciences Center.

A. Toxicity Studies on Freshwater-Reared Finfish Eggs

1. Freshwater Reared Finfish Eggs – Study TOX – 94 -00048 – 3

Title: Safety of Hydrogen Peroxide to Non-Eyed and Eyed Rainbow Trout *Oncorhynchus mykiss* Eggs

Study Director: Jeffrey J. Rach

Study Location: US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To investigate the toxicity of hydrogen peroxide treatments on rainbow trout non-eyed and eyed eggs at up to ten times the proposed dose (500 to 1000 mg/L) and for up to three times the proposed duration of treatment (15 minutes).

b. Animals: Two lots of fertilized non-eyed and eyed eggs were used in the study. Lot # 9403 was cultured at 12 °C, and lot # 9452 was cultured at 15 °C.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: A 30 mL volume of eggs containing approximately 197 to 312 eggs was randomly assigned to each test jar. Two lots of eggs were used. Hydrogen peroxide was administered at 0, 1130, 3390, 5650, and 11300 mg/L of water (approximately 0X, 1X, 3X, 5X, and 10X the highest proposed label dose) for 15 or 45 minutes (1X and 3X the treatment duration) every other day until the eggs became eyed (non-eyed egg treatments) or until eggs hatched (eyed egg treatments). The details of exposure time, egg lots and temperature of the water are presented in Table 11.

Treatments were terminated 13 days post-fertilization for eggs incubated at 12 °C, and 10 days post-fertilization for eggs incubated at 15 °C.

Table 11. Hydrogen peroxide treatment groups of two lots of rainbow trout fertilized eggs treated at 15 or 45 minutes every other day.

Group #	Egg Lot #	Temperature (+/- 2 °C)	Treatment Duration (Minutes)	# Treatments	Egg Stage at Test Initiation	Egg Age (days)	Final Life Stage
1	9403	12	15	6	Non-eyed	2	Eyed eggs
2	9403	12	45	6	Non-eyed	2	Eyed eggs
3	9452	15	15	4	Non-eyed	2	Eyed eggs
4	9452	15	45	4	Non-eyed	2	Eyed eggs
5	9403	12	15	5	eyed	14	Fry
6	9403	12	45	5	eyed	14	Fry
7	9452	15	15	3	eyed	14	Fry
8	9452	15	45	3	eyed	14	Fry

e. Variables Measured: The total number of dead and live eggs and fry at the termination of each trial was enumerated by direct count. Water flow was monitored daily and was turned off during hydrogen peroxide exposure. Water temperature, dissolved oxygen, pH, alkalinity, and hardness were measured during the study. Hydrogen peroxide concentrations were analyzed using a permanganate titration method.

f. Statistical Analysis: Survival of the non-eyed eggs to eye-up or eyed eggs to hatch was analyzed using a general linear model ANOVA with binomial distribution and logit link at the 0.10 level of significance. Incubation vessels were the experimental unit.

g. Results: Survival of non-eyed eggs to eye-up is illustrated in Table 12, and survival of eyed eggs to hatch is illustrated in Table 13.

Table 12. Mean percent eye-up of two lots of non-eyed rainbow trout eggs treated with hydrogen peroxide every other day for 15 or 45 minutes until eggs reached the eyed-egg stage at incubation temperatures of 12 or 15 +/- 2 °C.

Temperature °C	Time Minutes	Lot #	Treatment Concentration (mg/L)				
			0	1130	3390	5650	11300
			Mean eye-up (%)				
12	15	9403	96	86*	54*	59*	42*
12	45	9403	96	48*	16*	4*	NT
15	15	9452	76	57*	39*	33*	10*
15	45	9452	76	26*	10*	5*	NT

NT: Not tested
* Significantly different from control ($p \leq 0.10$)

Table 13. Mean percent hatch of two lots of eyed rainbow trout eggs treated with hydrogen peroxide every other day for 15 or 45 minutes at incubation temperatures of 12 or 15 +/- 2 °C until hatch.

Temperature °C	Time Minutes	Lot #	Treatment Concentration (mg/L)				
			0	1130	3390	5650	11300
			Mean hatch (%)				
12	15	9403	90	97*	95*	97*	97*
12	45	9403	90	95*	92	95*	NT
15	15	9452	90	88	88	89	85*
15	45	9452	90	86*	85[1]	84*	NT

NT: Not tested
* Significantly different from control ($p \leq 0.10$)
[1] Not significant because of large standard error

Water flow was adjusted to 300 mL/minute between treatments. Temperatures ranged from 12.1 to 12.9 °C and 14.5 to 15.2 °C. Dissolved oxygen ranged from 8.9 to 20.0 mg/L; pH ranged from 7.0 to 8.3; alkalinity ranged from 97 to 102 mg/L; and water hardness ranged from 140 to 142 mg/L. Hydrogen peroxide concentrations in the water were within acceptable ranges (+/- 15% of the target concentrations).

The probability of eye-up was significantly less in the treated non-eyed eggs than in the control eggs. The probability of hatch was significantly less in control eggs than in treated eyed eggs that were incubated at 12 °C, except for the 3X concentration group with 45-minute treatments which showed a numerical difference. The probability of hatch was not significantly different when eyed eggs incubated at 15 °C were treated at doses up to 5X the highest label dose in 15-minute applications compared to untreated controls. When eyed eggs incubated at 15 °C were treated with hydrogen peroxide for 45 minutes every other day, the probability of hatch was always less, sometimes significantly, in the treated groups than in the control.

h. Conclusions: This study demonstrates the safety of hydrogen peroxide at 1000 mg/L for 15 minutes daily until hatch on eyed rainbow trout eggs. An adequate margin of safety exists above 1130 mg/L for hydrogen peroxide treatment of eyed rainbow trout eggs for 15 minutes every other day. Hydrogen peroxide treatment at 1130 mg/L or higher for 15 minutes every other day from egg fertilization to eye-up decreased the probability of egg eye-up. See further investigation in Section III.A.4.

2. Target Animal Safety Study – No. TOX-95-00048-7 (1995 – 1996) – Report No. 2

Title: Safety of Hydrogen Peroxide Treatments on Fish Eggs

Study Director: Jeffrey J. Rach

Study Location: US Geological Survey
Upper Mississippi Science Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the safety of hydrogen peroxide as a 15-minute water treatment at concentrations of 566, 1132, 3396, and 6792 mg/L daily until hatching of fertilized eggs. These concentrations approximate the low end of the label dose range and 1X, 3X, and 6X the high end of the label dose range.

b. Animals: One to three-day old fertilized eggs were used; Table 14 lists the species tested.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study. Hydrogen peroxide was administered in a continuous flow system.

d. Study Design: Each aquarium (for catfish and perch) or jar received 30 mL of eggs. Eggs of walleye (*Stizostedion vitreum*), yellow perch (*Perca flavescens*), white sucker (*Catostomus commersonii*), lake sturgeon (*Acipenser fulvescens*), paddlefish (*Polyodon spathula*), common carp (*Cyprinus carpio*), and channel catfish (*Ictalurus punctatus*) were treated with hydrogen peroxide at 0, 1132, 3396, and 6792 mg/L. Eggs of northern pike (*Esox lucius*) were allocated in the same manner and treated with hydrogen peroxide at 0, 566, 1132, and 3396 mg/L. Treatments were initiated when the eggs were 1 to 3 days old and were administered for 15 minutes once daily (Monday through Friday) until all viable eggs hatched. There were three replicates for each concentration.

   This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables measured: Mean percent hatch and water flow, temperature, dissolved oxygen, pH, and hardness. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Percent hatch was calculated by dividing the number of fry by the initial number of eggs and multiplying by 100.

g. Results: Hatch results are summarized in Table 14.

Water hardness ranged from 142 to 160 mg/L $CaCO_3$; alkalinity ranged from 101 to 138 mg/L $CaCO_3$. Hydrogen peroxide concentrations in the test tanks were within 5% of the target concentrations. Water flow rate was 360 +/- 36 mL/minute in all tanks. Water temperature was 12 +/- 2 °C for eggs of northern pike, walleye, yellow perch and white sucker; 17 +/- 2 °C for lake sturgeon, paddlefish, and carp; and 22 +/- 2 °C for channel catfish. Dissolved oxygen was maintained between 7.4 to 20.0 mg/L. Water pH ranged from 7.89 to 8.72.

Table 14. Mean percent hatch of fish eggs treated with hydrogen peroxide.

	Treatment Groups (mg/L)				
Species	0 mg/L	566 mg/L	1132 mg/L	3396 mg/L	6792 mg/L
Northern pike	32	32	37	34	-[a]
Walleye	0[b]	-[a]	77	61	5
Yellow perch	59[b]	-[a]	100[c]	66	18
White sucker	15[b]	-[a]	61	42	0
Lake sturgeon	51[b]	-[a]	57[c]	61[c]	40
Paddlefish	72[b]	-[a]	82[c]	53	42[d]
Common carp	6[b]	-[a]	59	53	48
Channel catfish	19[b]	-[a]	78	68	0

a. Not tested
b. Fungus observed on eggs
c. One replicate not used due to aquarium overflow (n=2)
d. All fish died after post-hatch treatments

Hydrogen peroxide concentrations were within 5% of the target concentrations.

h. Conclusions: This study demonstrates an adequate margin of safety for hydrogen peroxide above 1132 mg/L for treatments administered for 15 minutes daily on northern pike, lake sturgeon, and common carp. The margin of safety above 1132 mg/L for walleye, yellow perch, white sucker, paddlefish, and channel catfish could not be determined from this study. The effect of treatment was confounded by variable fungal infection of the treated and control eggs in this study.

3. Freshwater-Reared Finfish Eggs – Study TOX – 94 -00048 – 2, Part 1

Title: Hydrogen Peroxide Treatment Toxicity to Rainbow Trout Eggs, Part 1: Safety

Study Director: Jeffrey J. Rach

Study Location: Upper Mississippi Science Center
US Geological Survey
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the safety of hydrogen peroxide at 566, 1132, and 3396 mg/L for 15 minutes daily from fertilization until 5 days after hatch on eggs and fry of rainbow trout and steelhead trout. These concentrations approximate the low end of the label dose range and 1X and 3X the high end of the label dose range.

b. Animals: Five lots of non-eyed 36-hour-old fertilized rainbow trout (*Oncorhynchus mykiss*) eggs were used in the study. Lots 9624, 9653, and 9658 were rainbow trout (75,000 eggs); lot 9604 (5,000 eggs) was rainbow trout steelhead strain Skamania; and lot 9621 was rainbow trout steelhead strain Ganaraska. Control and treated eggs from all lots tested remained free of fungal infection throughout incubation.

c. Test Articles: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: A volume of 30 mL of eggs of each strain was assigned to each experimental group. The suspension included approximately 148 to 273 eggs. Three replicates were included in the study. Hydrogen peroxide was delivered using a peristaltic pump at 0, 566, 1132, and 3396 mg/L for 15 minutes daily (Monday through Friday) from fertilization until 5 days after hatch.

e. Variables: Percent hatch was calculated by dividing the number of fry (live and dead) by the total number of eggs and multiplying by 100. Water flow, temperature, dissolved oxygen, pH, and alkalinity were measured during the study. Hydrogen peroxide concentrations were verified analytically using a permanganate titration method.

f. Statistical Analysis: Percent hatch was calculated by dividing the number of fry (live and dead) by the total number of eggs and multiplying by 100.

g. Results: The percent hatch for each treatment is summarized in Table 15.

Water temperature was 12 +/- 2 °C; dissolved oxygen was 9.0 to 20.0 mg/L; and water pH ranged from 7.88 to 8.13. Hydrogen peroxide exposure concentrations were within +/- 10% of the target concentrations.

Table 15. Mean percent hatch of rainbow trout and steelhead trout eggs after exposure to hydrogen peroxide treatment for 15 minutes daily from fertilization until 5 days after hatch.

	Hydrogen Peroxide mg/L Mean Hatch (%)			
Eggs Lot Number	**0**	**566**	**1132**	**3396**
9614 Rainbow trout	94.1	92.1	86.7	79.0
9653 Rainbow trout	78.4	77.7	71.6	65.2
9658 Rainbow trout	83.1	77.2	67.7	57.8
9604 Skamania steelhead	73.6	49.6	28.7	28.0
9621 Ganaraska steelhead	79.1	72.6	73.5	3.5

h. Conclusions: This study does not demonstrate the safety of hydrogen peroxide on rainbow trout eggs. There is a numerical decrease in mean percent hatch as the concentration of hydrogen peroxide increases. Steelhead trout eggs are more sensitive to treatment than rainbow trout eggs. A statement is needed on the label which recommends that users conduct bioassays on a small number before treating the entire group to discern potential species-specific sensitivities. Further investigations with rainbow trout eggs revealed a sensitive period during incubation between 70 and 140 Daily Thermal Units degrees Celsius; see Sections III.A.4 and III.A.5.

4. Freshwater-Reared Finfish Eggs – Study CAP-96-00048-2, Part 2

Title: Hydrogen Peroxide Treatment Toxicity to Rainbow Trout Eggs, Part 2: Identification of the Sensitive Period During the Exposure of Rainbow Trout Eggs to Hydrogen Peroxide

Study Director: Mark P. Gaikowski

Study Location: US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To assess the sensitivity of rainbow trout embryos to hydrogen peroxide at 0 (control), 566, 1132, and 3396 mg/L of water for a 15-minute daily treatment from 1 to 3 days post-fertilization ($\leq$ 20 Daily Thermal Units degrees Celsius (DTU °C)) through hatch. These concentrations approximate the low end of the label dosage range and 1X and 3X the high end of the label dosage range.

b. Animals: A total of 75,000 rainbow trout eggs were used in the study. They were obtained from three different geographic locations (Lots 9628, 9653, and 9658). Treatments were initiated when eggs were 1 to 3 days post-fertilization. Eggs remained free of fungus throughout incubation.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Four treatments with three replicates each were established in the study. Hydrogen peroxide was tested at 0, 566, 1132, and 3396 mg/L as an immersion treatment in a continuous flow system for 15 minutes daily every weekday (Monday through Friday) until all eggs hatched. Eggs were added to each tank as a 30 mL volume, containing approximately 203 to 274 eggs.

This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables Measured: Dead eggs were removed daily from aquaria to calculate the mean daily cumulative percent mortality. The day after all viable eggs hatched, all hatched fry (live and dead) and dead eggs were counted. Water flow rate, temperature, dissolved oxygen, and pH were measured and recorded during the study. Water samples were collected during treatment exposure, and hydrogen peroxide concentrations were analyzed using a permanganate titration method.

f. Statistical Analysis: Mean cumulative daily percent mortality was calculated for non-statistical comparisons between days within treatments. Embryo sensitivity

was compared using numbers of live and dead eggs recorded during a given 5-day period. Embryo sensitivity was determined by identifying a time interval when the hazard of death was maximized.

g. Results: The hatch rates of treated and control groups are summarized in Table 16.

Table 16. Mean percent hatch of rainbow trout eggs treated with hydrogen peroxide for 15 minutes daily (Monday through Friday). All viable eggs had hatched by Day 25 or 27, depending on lot.

Egg Lot #	**Day**	**Hydrogen Peroxide (mg/L)** % Cumulative Mortality			
		0	**566**	**1132**	**3396**
9628	5	0.46	1.02	0.64	0.00
	10	6.43	25.68	20.71	47.19
	25	13.35	28.20	23.11	62.19
9653	5	0.39	10.00	1.22	0.25
	10	10.89	34.26	25.80	47.82
	27	26.03	39.31	33.41	57.10
9658	5	0.00	0.00	0.14	0.00
	10	8.70	25.84	46.24	75.44
	25	18.66	29.43	50.04	82.05

The sensitivity of rainbow trout eggs to hydrogen peroxide was greatest from Day 6 to Day 10, equivalent to 78 to 135 DTU °C.

Water flow was maintained at 300 +/- 30 mL/min. Water temperature ranged from 12 +/- 2 °C; dissolved oxygen ranged from 9.1 to 20.0 mg/L; and pH ranged from 7.92 to 8.15.

h. Conclusions: This study demonstrates that rainbow trout eggs are sensitive to daily 15-minute treatments with hydrogen peroxide at 566 to 1132 mg/L during Days 6 to 10 of development (78 to 135 DTU °C). Therefore, a limitation and caution regarding treatment of rainbow trout eggs at this stage of development is necessary on the label.

5. Freshwater-Reared Finfish Eggs – Study CAP-00-H2O2 – 1

Title: Safety of Hydrogen Peroxide to Paddlefish *Polyodon spathula* and Rainbow Trout *Oncorhynchus mykiss* eggs

Study Director: Jeffrey J. Rach

Study Location: US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To evaluate the safety of hydrogen peroxide exposure to paddlefish eggs and rainbow trout eyed and non-eyed eggs.

b: Animals: Paddlefish eggs and rainbow trout eggs were used in the study.

c. Test Article: Hydrogen peroxide (35% w/w) was used in the study.

d. Study Design: Hydrogen peroxide was administered as a continuous flow treatment; flow rate was 360 +/- 36 mL/minute. A 30 mL volume of eggs of each fish species was placed in each McDonald jar that was connected to an aquarium. The mean number of eggs per jar was 1,830 for paddlefish and 360 for rainbow trout. Each test had five treatment groups: 0 (control), 1000, 1500, 2000, and 2500 mg/L. These concentrations represent 0X, 1X, 1.5X, 2.0X and 2.5X the high end of the label dosage range, respectively. For paddlefish, there were 3 replicates for each treatment concentration and for rainbow trout, there were 6 replicates for each treatment concentration. Each group was treated for 15 minutes daily starting within 72 hours of fertilization and continuing daily until all viable eggs hatched. Paddlefish eggs were maintained at 17 +/- 2 °C, and rainbow trout eggs were incubated at 12 +/- 2 °C. Jars were protected from direct sun light. The duration of the study was 8 days for paddlefish and 26 to 27 days for rainbow trout.

This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

e. Variables Measured: At the end of the study, dead and live eggs and fry were counted. Water temperature, water alkalinity, water hardness, dissolved oxygen, and pH were monitored daily. Hydrogen peroxide concentrations in each group were verified analytically using a permanganate titration method.

f. Statistical Analysis: Logit transformed hatch rates were analyzed using a generalized linear model ANOVA based on the SAS procedure GENMOD.

Treatment comparisons were made at the 0.10 level of significance using least-square means.

g. Results: Mean percent hatch of treated eggs is summarized in Table 17.

Table 17. Mean percent hatch of paddlefish and rainbow trout eggs treated with hydrogen peroxide for 15 minutes daily.

	Treatment Groups (mg/L) Hatch (%)				
Species	**0**	**1000**	**1500**	**2000**	**2500**
Rainbow trout	75.7^{a}	52.2^{b}	47.5^{bc}	44.0^{c}	34.5^{d}
Paddlefish	7^{a}	35^{b}	26^{b}	27^{b}	25^{b}

^{a,b,c,d} Means with different superscripts within a species differ at the 0.10 level of significance.

Water temperature ranged from 16.9 to 18.5 °C for paddlefish eggs, and 12.2 to 15.0 °C for rainbow trout eggs. The pH ranged from 7.37 to 8.22 for paddlefish eggs and 7.67 to 8.14 for rainbow trout eggs. Alkalinity ranged from 107 to 138 mg/L (as $CaCO_3$) and hardness from 140 to 162 mg/L (as $CaCO_3$). Dissolved oxygen concentrations ranged from 4.4 (due to one deviation) to greater than 20 mg/L for paddlefish eggs and from 8.0 to 18.0 mg/L for rainbow trout eggs. The mean hydrogen peroxide concentrations for all tests were within 15% of anticipated concentrations.

h. Conclusions: This study demonstrates a margin of safety of hydrogen peroxide above the highest proposed dose, 1000 mg/L for 15 minutes daily until hatch, on paddlefish eggs, because there was no significant decrease in percent hatch of paddlefish eggs between concentrations 1000 and 2500 mg/L. The mortality in rainbow trout eggs in this study is similar to that observed in the study in Section III.A.4, supporting the need for a limitation and caution statement on the label.

B. Toxicity Studies on Freshwater-Reared Finfish:

1. Target Animal Safety Study – No. CAP-97-00048-08

Title: Toxicity Assessment of Hydrogen Peroxide to Cold-, Cool-, and Warmwater Fish

Study Director: Mark P. Gaikowski

Study Locations: U.S. Geological Survey
a. Upper Midwest Environmental Sciences Center
La Crosse, WI
b. Kearneysville, WV

General Design of the Study:

a. Purpose: To determine the species most sensitive to 60- and 180-minute hydrogen peroxide bath treatments by testing fry and/or fingerlings of one or more fish species from each of the major families cultured by public aquaculture (Salmonidae, Esocidae, Percidae, Ictaluridae, Catostomidae, Centrarchidae, Cyprinidae, Percichthyidae, Acipenseridae, and Polyodontidae).

b. Animals/Controls: For all fish species, fingerlings tested were at least one month older at study initiation than the fry tested. The following species were tested: rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar*), northern pike (*Esox lucius*), muskellunge (*Esox masquinongy*), walleye (*Stizostedion vitreum*), yellow perch (*Perca flavescens*), channel catfish (*Ictalurus punctatus*), large mouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), white sucker (*Catostomus commersonii*), fathead minnow (*Pimephales promelas*), pallid sturgeon (*Scaphirhynchus albus*), and paddlefish (*Polyodon spathula*). Fish were obtained from various state and federal hatcheries. Test fish were maintained at appropriate temperatures and culture conditions for their species and life stage. Test fish were fed a diet appropriate for their species and life stage.

c. Study Design: There were six groups (five treated and one non-treated control) for each species and life stage. The drug concentrations for the 60- and 180-minute exposure times varied depending on the results of an initial range-finding study. Dose ranges were selected to include therapeutic and lethal concentrations. Fish were exposed to a total of three static baths of hydrogen peroxide administered every other day. Each treatment group was replicated 3 times. Fish were observed for mortality for 96 hours after the last exposure. Masking procedures were followed for allocation of fish to tanks, water sampling, dose verification, mortality recording, and gill histology evaluation. Well water was used in all tanks and was kept at 12 +/- 2 °C, 17 +/- 2 °C, or 22 +/- 2 °C

depending upon the fish species. Table 19 summarizes fish species and life stages and treatment concentrations and durations tested.

This study was conducted in accordance with Good Laboratory Practices (21 CFR 58).

d. Variables Measured: Mortality was recorded at 0, 24, 48, 72, 96, 120, 144, 168, and 192 hours after the first treatment. Mortality was defined as the cessation of opercular movement and lack of response to gentle prodding. At 96 and 192 hours, three fish were removed from the control tank and from each of the tanks testing the three lowest hydrogen peroxide concentrations and necropsied; the second gill arch was collected and fixed in 2.5% glutaraldehyde – 2% formaldehyde solution. Samples from the 60-minute time point were examined histologically.

Dissolved oxygen, temperature, and pH were recorded daily for each tank. For each treatment, hydrogen peroxide concentration was verified by permanganate titration.

e. Statistical Analysis: Mean cumulative percent mortality was calculated for non-statistical comparisons between treatments.

f. Results: Fish mortality and concentrations of hydrogen peroxide tested are summarized in Table 19.

Mean dissolved oxygen concentrations transiently increased during treatment exposure and ranged from 9.9 to greater than 20 mg/L during exposure at 12 +/ 2 °C, 7.2 to 18.1 mg/L at 17 +/- 2 °C, and 7.4 to greater than 20 mg/L at 22 +/- 2 °C. Water quality parameters observed during the remainder of the study are summarized in Table 18. Hydrogen peroxide concentrations were expected to be within +/- 10% of target concentrations; deviations were reported and considered not to impact the final conclusion.

Table 18. Water quality parameters in Study No. CAP-97-00048-08.

Culture System	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
12 +/- 2 °C	12.4 to 14.3	9.5 to 10.0	7.59 to 8.23
17 +/- 2 °C	16.7 to 18.0	7.6 to 9.6	7.37 to 8.20
22 +/- 2 °C	20.1 to 22.3	7.2 to 8.7	7.56 to 8.33

Table 19. Results: Fish species and life stages, mean percent mortality after treatment, hydrogen peroxide concentrations, and exposure times.

Species	Mean Mortality (%) / Hydrogen peroxide Conc. (mg/L)	Exposure time
Atlantic salmon fingerlings	7/0, 7/150, 0/250, 53/417, 100/695, 100/1158	60 minutes
	0/0, 0/49, 0/81, 0/136, 67/226, 100/376	180 minutes
Rainbow trout fry	0/0, 7/212, 67/354, 100/706, 100/1413, 100/2825	60 minutes
	0/0, 20/88, 87/147, 100/244, 100/407, 100/678	180 minutes
Rainbow trout fingerlings	0/0, 4/183, 12/305, 96/509, 100/848, 100/1413	60 minutes
	0/0, 0/92, 58/153, 100/254, 100/424, 100/706	180 minutes
Lake trout fingerlings	0/0, 13/337, 100/562, 100/936, 100/1559, 100/2599	60 minutes
	0/0, 0/46, 0/77, 13/128, 67/212, 100/354	180 minutes
Northern pike fry	30/0, 27/111, 50/185, 93/310, 100/515, 100/859	60 minutes
	17/0, 33/36, 30/61, 62/102, 100/170, 100/283	180 minutes
Northern pike fingerlings	4/0, 37/86, 100/144, 100/238, 100/398, 100/663	60 minutes
	0/0, 12/36, 75/61, 100/102, 100/170, 100/283	180 minutes
Muskellunge fry	4/0, 0/118, 62/195, 71/325, 100/542, 100/904	60 minutes
	0/0, 0/36, 8/61, 88/102, 100/170, 100/283	180 minutes
Muskellunge fingerlings	0/0, 0/118, 25/195, 75/325, 100/542, 100/904	60 minutes
	0/0, 0/53, 0/88, 29/147, 100/244, 100/407	180 minutes
Walleye fry	0/0, 0/29, 0/49, 20/81, 67/136, 100/226	60 minutes
	0/0, 0/18, 0/29, 0/49, 7/81, 100/136	180 minutes
Walleye fingerlings	4/0, 0/24, 0/40, 0/66, 20/108, 93/181	60 minutes
	8/0, 0/11, 4/19, 0/32, 13/53, 80/88	180 minutes
Yellow perch fry	0/0, 13/32, 13/53, 40/88, 100/147, 100/244	60 minutes
	0/0, 7/10, 0/17, 20/28, 7/47, 60/79	180 minutes
Yellow perch fingerlings	0/0, 0/88, 12/147, 71/244, 93/407, 100/678	60 minutes
	0/0, 0/53, 0/88, 50/147, 93/244, 100/407	180 minutes
Channel catfish fry	0/0, 0/53, 7/88, 87/147, 100/244, 100/407	60 minutes
	0/0, 0/19, 0/32, 31/53, 100/88, 100/147	180 minutes
Channel catfish fingerlings	0/0, 0/53, 17/88, 87/147, 100/244, 100/407	60 minutes
	0/0, 0/19, 0/32, 12/53, 100/88, 100/147	180 minutes
Largemouth bass fry	0/0, 0/121, 0/202, 67/337, 100/562, 100/936	60 minutes
	0/0, 0/103, 93/171, 100/285, 100/475, 100/791	180 minutes
Largemouth bass fingerlings	4/0, 0/53, 4/88, 8/147, 87/244, 67/407	60 minutes
	0/0, 0/53, 42/88, 100/147, 100/244, 100/407	180 minutes
Bluegill fry	0/0, 0/53, 0/88, 80/147, 100/244, 100/407	60 minutes
	0/0, 0/32, 0/53, 40/88, 100/147, 100/244	180 minutes
Bluegill fingerlings	0/0, 0/32, 0/53, 12/88, 77/147, 67/244	60 minutes
	0/0, 0/32, 0/53, 87/88, 100/147, 100/244	180 minutes
White sucker fry	7/0, 0/53, 60/88, 100/147, 100/244, 100/407	60 minutes
	7/0, 7/19, 7/32, 33/53, 93/88, 100/147	180 minutes
White sucker fingerlings	0/0, 4/88, 67/147, 100/244, 100/407, 100/678	60 minutes
	0/0, 4/53, 73/88, 100/147, 100/244, 100/407	180 minutes
Fathead minnow fry	0/0, 0/19, 0/32, 0/53, 60/88, 100/147	60 minutes
	0/0, 0/11, 0/19, 0/32, 93/53, 100/88	180 minutes
Fathead minnow fingerlings	0/0, 0/53, 12/88, 96/147, 100/244, 100/407	60 minutes
	0/0, 0/32, 0/53, 96/88, 100/147, 100/244	180 minutes
Pallid sturgeon fry	4/0, 67/129, 67/215, 100/366, 100/610, 100/1017	60 minutes
	0/0, 4/32, 50/53, 100/88, 100/147, 100/244	180 minutes
Pallid sturgeon fingerlings	0/0, 4/105, 42/175, 100/293, 100/488, 100/814	60 minutes
	0/0, 0/32, 0/53, 96/88, 100/147, 100/244	180 minutes
Paddlefish fingerlings	0/0, 73/73, 100/122, 100/203, 100/339, 100/565	60 minutes
	Not tested	180 minutes

g. Conclusions: This study demonstrates an adequate margin of safety for the use of hydrogen peroxide for the following dosing tiers: Up to 100 mg/L for 60 minutes in a static bath or for 30 minutes in a continuous flow system are safe for freshwater-reared salmonids, largemouth bass, and muskellunge. For fingerlings, static bath treatments up to 75 mg/L for 60 minutes are safe for pallid sturgeon, walleye, white sucker, bluegill, channel catfish, fathead minnow, yellow perch, and all other freshwater-reared finfish (except northern pike and paddlefish). For fry, static bath treatments up to 50 mg/L for 60 minutes are safe for walleye, white sucker, bluegill, channel catfish, fathead minnow, yellow perch, and all other freshwater-reared finfish (except northern pike, paddlefish, and pallid sturgeon). Hydrogen peroxide is not safe for use in pallid sturgeon fry or any non-egg life stages of northern pike or paddlefish. A preliminary bioassay should be done to determine species and life stage sensitivity before treating a large group of fish.

IV. HUMAN FOOD SAFETY:

The human food safety of the use of hydrogen peroxide on all species and life stages of fish, including eggs, has been met by safety evaluations completed by several groups: the Select Committee on Generally Recognized as Safe (GRAS) Substances, FDA (48 FR 52323), and the U.S. Environmental Protection Agency (EPA) (63 FR 24955). Also considered in the human food safety evaluation were the dosing regimes for finfish and finfish eggs, and the fact that hydrogen peroxide is a normal product of aerobic metabolism, decomposing to oxygen and water in the absence of a stabilizing agent.

The Select Committee was chosen by the Life Sciences Research Office of the Federation of American Societies for Experimental Biology (FASEB) for FDA's proposed affirmation that hydrogen peroxide is GRAS, with specific limitations, as a direct human food ingredient. The Select Committee determined that specific uses of hydrogen peroxide are GRAS after a consideration that most of the chemical is destroyed or dissipated during processing and that there is no nutritionally significant destruction of essential nutrients from the use of hydrogen peroxide. In addition, there was no evidence that hydrogen peroxide is carcinogenic, teratogenic, or mutagenic at levels present in foods treated with hydrogen peroxide during processing. Also, none of the oxidation products formed by action of hydrogen peroxide on food constituents were proven to be carcinogenic when given by mouth.

FDA concurred with the conclusion of the Select Committee and conducted its own evaluation of the safety of hydrogen peroxide using all available information on hydrogen peroxide, including information not available to the Select Committee. In addition, FDA determined that there is not sufficient evidence to conclude that hydrogen peroxide is a duodenal carcinogen (memorandum of FDA's Cancer Assessment Committee meetings on this matter is on file under Docket No. 78N-0369).

EPA completed a risk assessment to support an exemption from the requirement of a tolerance for residues of the antimicrobial pesticide hydrogen peroxide up to 120 ppm, in or on raw agricultural commodities, in processed commodities, when such residues result from the use of hydrogen peroxide as an antimicrobial agent on fruits, tree nuts, cereal grains, herbs, and spices. At the low proposed use concentrations, no residues of toxicological concern were expected on any animal feeds that may be exposed to hydrogen peroxide and no residues of toxicological concern were anticipated either in animals that may consume these feeds, or in associated animal by-products.

The dosing regime for hydrogen peroxide on finfish and their eggs ensures that animals will not remain in contact with hydrogen peroxide indefinitely. Fish and their eggs will be exposed to water containing hydrogen peroxide for a finite period of time. After the treatment period is over, the fish and their eggs will either be removed from the treated water or the treated water will be flushed out and replaced with untreated water.

Any hydrogen peroxide remaining on the finfish or eggs will degrade into oxygen and water which are compounds of no toxicological concern. The small amount of hydrogen peroxide that could be ingested by humans will most likely decompose in the gastrointestinal tract, leaving little intact compound for absorption thereby posing a low risk for the development of hydrogen peroxide resistance and any associated antimicrobial resistance in foodborne pathogens of human health concern, including *Escherichia coli*, *Salmonella* and *Campylobacter*. Any absorbed hydrogen peroxide would be rapidly decomposed by tissue catalase or peroxidase to oxygen and water and would probably not cause any adverse effect on the human intestinal flora.

The human food safety concerns for the use of hydrogen peroxide on all finfish and their eggs are satisfied. Neither an acceptable daily intake (ADI), tolerance, withdrawal time, nor regulatory methods are assigned.

V. USER SAFETY:

User safety was evaluated by reviewing the Material Safety Data Sheet and product fact sheet and incorporating appropriate information on the product label. The product label contains the following information regarding safety to humans handling, administering, or exposed to 35% PEROX-AID:

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. 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.

INHALATION (Breathing):
Avoid breathing vapor or mist; causes irritation of the nose, throat, and lungs; overexposure 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 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

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 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 finfish eggs due to saprolegniasis, for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*, and for the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *Flavobacterium columnare (Flexibacter columnaris)*. Additionally, data demonstrate that residues in food products derived from finfish 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:

Under section 573(c) of the Federal Food, Drug and Cosmetic Act (the Act), this approval qualifies for SEVEN years exclusive marketing rights beginning on the date of approval because the new animal drug has been declared a designated new animal drug by FDA under section 573(a) of the Act.

C. Patent Information:

The sponsor did not submit any patent information with this application.

VII. ATTACHMENTS:

Facsimile Labeling:

55-gallon container label
5-gallon container label
Product data sheet