Date of Approval: April 11, 2019

# FREEDOM OF INFORMATION SUMMARY

## SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 140-989

PARASITE-S

formalin

Concentrated immersion solution

Freshwater-reared finfish

For the control of mortality in freshwater-reared finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae

Sponsored by:

Syndel USA

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# I. GENERAL INFORMATION

## A. File Number

NADA 140-989

## B. Sponsor

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

Drug Labeler Code: 050378

## C. Proprietary Name

PARASITE-S

## D. Drug Product Established Name

Formalin

## E. Pharmacological Category

Fungicide

## F. Dosage Form

Concentrated immersion solution

## G. Amount of Active Ingredient

37% (by weight) formaldehyde

## H. How Supplied

1-gallon, 5-gallon, 55-gallon, and 1000-Liter containers

## I. Dispensing Status

Over-the-counter (OTC)

## J. Dosage Regimen

150 milligrams per liter water for a one-hour bath every other day for three treatments

## K. Route of Administration

Immersion

## L. Species/Class

Freshwater-reared finfish

### M. Indication

For the control of mortality in freshwater-reared finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae

### N. Effect of Supplement

This supplement provides for the addition of the following indication: For the control of mortality in freshwater-reared finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae.

## II. EFFECTIVENESS

The effectiveness of PARASITE-S was demonstrated in two induced infection model studies described below. These studies demonstrated that PARASITE-S is effective in controlling mortality associated with saprolegniasis in rainbow trout and channel catfish, two representative freshwater finfish species, at both 100 mg/L and 150 mg/L for a 1-hour bath every other day for three treatments. The results of those studies, along with a body of evidence of use of PARASITE-S in natural outbreaks, supports a dose of 150 mg/L for a 1-hour bath every other day for three treatments. The induced infection model studies were conducted at FDA Center for Veterinary Medicine, Office of Research, and are contained in the publicly disclosable INAD file 11-365 sponsored by the U.S. Geological Survey, Upper Midwest Environmental Sciences Center and are also summarized in PMF 5228.

### A. Dosage Characterization

Formalin is a water treatment where the primary effect results from localized action at the topical site of administration. The concentration of 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. PARASITE-S is currently approved for the control of fungi of the family Saprolegniaceae on finfish eggs. The formalin doses selected for the effectiveness trials were based on toxicity studies and hatchery trials in freshwater-field finfish. Based on that information, formalin administered at doses of 50 mg/L, 100 mg/L, and 150 mg/L for 60 minutes on alternate days for three treatments had the potential to control mortality due to saprolegniasis associated with fungi in the family Saprolegniaceae in freshwater-reared finfish.

### B. Substantial Evidence

1. Induced Infection Model Study

   **Title:** Efficacy of Formalin for Treating Fungal Infections in Rainbow Trout. Study No. 401-07.

   **Study Dates:** November 26, 2002, to October 14, 2004

   **Study Location:** Laurel, Maryland, U.S.A.

**Study Design:**

Objective: To evaluate formalin at concentrations of 0, 50, 100, and 150 mg/L to control mortality due to *Saprolegnia* infections (saprolegniasis) in rainbow trout (*Oncorhynchus mykiss*). This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

Study Animals: Two hundred (200) rainbow trout fingerlings were selected for the study. The study fish had a mean length of 16.5 (14.5 to 19.5) centimeters and a mean body weight of 46.9 (31.4 to 71.0) grams.

Experimental Design: Four trials were performed. For each trial, 50 fingerlings were used (48 experimental fish plus 2 extras). The fish were anesthetized with tricaine methanesulfonate and lightly abraded above the lateral line between the posterior margin of the dorsal fin and the anterior margin of the adipose fin using a mechanical scarification technique. The fish recovered from anesthesia before being placed into the exposure tank. Temperature stress was performed concurrently with fungal exposure by moving the trout from the acclimation temperature (15 ± 2 °C) to a higher exposure temperature (22 ± 2 °C). The fish were immersed together for 4 hours in a single bath exposure tank which had been inoculated with culture of *Saprolegnia parasitica*. After exposure, fish were randomly distributed into individual compartments within 16 experimental (treatment) tanks (3 fish/tank). Each tank was randomly assigned to one of the treatment groups (0, 50, 100, and 150 mg/L formalin). Individual tank is the experimental unit.

Drug Administration: Treatments of 0, 50, 100, and 150 mg/L formalin were administered once daily every other day starting one hour after exposure ended for a total of three treatments. Each treatment lasted one hour. Fish were held for observation for two weeks post-treatment.

Measurements and Observations: All fish were monitored twice daily during the treatment and post-treatment observation period. A diagnosis of saprolegniasis was made visually based on the clinical manifestation of colony morphology on the fish. Moribund and dead fish were removed daily, examined grossly (the length and width of the lesion was measured, and the depth of the lesion was evaluated), and selected fish had tissues harvested for necropsy and histology. After the observation period, all surviving fish were euthanized, examined, and processed similarly to the dead and moribund fish. Water temperature, pH, and dissolved oxygen were recorded throughout the trials. Water samples, to verify formalin concentrations in the treatment tanks, were taken from the treatment water just before ending the 1-hour treatments.

**Statistical Methods:** Cumulative percent mortality on Days 5 and 19 was analyzed with the generalized linear models procedure available in "PROC GENMOD" in SAS version 8.2. The analysis was done with the fixed effects treatment, trial, and the treatment by trial interaction in the model. Treatment was tested at the $\alpha = 0.05$ significance level. If treatment was significant, follow-up pairwise mean comparisons between the control group

and treated groups were performed using linear contrasts at significance level $\alpha = 0.05$.

**Results:** Cumulative mortality results are included in Tables 1 and 2. The majority of mortalities occurred by Day 5 in all trials. In the analyses for both Days 5 and 19, the interaction was not significant (P = 0.4832 and 0.5274, respectively), so the data were re-analyzed with only the main effects treatment and trial in the model. The treatment effect was significant for both Days 5 and 19 (P = 0.0004 and 0.0007, respectively), so follow-up comparisons were made. For each day, every treatment group had a significantly lower mortality rate than the control group.

The dissolved oxygen (DO) ranged from 6.2 to 10.6 mg/L. The pH ranged from 7.13 to 7.84. The water temperature of the exposure tanks ranged from 20 to 24 °C while the temperature of the treatment tanks was maintained around 16 °C. The water quality results were acceptable for the culture of rainbow trout.

Clinical manifestations of the fungus on the fish (hyphae and fluffy growths on the skin, fins, gills, or eyes) 24 hours after exposure were used to verify the presence of infective fungus in the tank. Prominent fungal growth was seen in all fish. Histology on dead and moribund fish confirmed the gross diagnosis. Fungal hyphae were seen penetrating through the connective tissue of the dermis in some specimens. In surviving fish, the large fungal mats would slough and leave a red lesion which would typically contract. As the lesions healed, they became darkly pigmented. Histologically, a moderate to marked hyperplastic epithelium with some areas of angiogenesis covered the lesions in most surviving fish.

**Table 1: Cumulative mortality on Days 5 and 19 following exposure.**

| Day | Dose (mg formalin /L) | Trial 1 | Trial 2 | Trial 3 | Trial 4 |
|---|---|---|---|---|---|
| 5 | 0 | 5 | 7 | 9 | 10 |
| 5 | 50 | 4 | 3 | 3 | 7 |
| 5 | 100 | 2 | 5 | 2 | 4 |
| 5 | 150 | 3 | 3 | 3 | 9 |
| 19 | 0 | 5 | 7 | 9 | 11 |
| 19 | 50 | 4 | 3 | 3 | 7 |
| 19 | 100 | 3 | 5 | 2 | 4 |
| 19 | 150 | 3 | 4 | 3 | 9 |

**Table 2: Mean percent mortality for each treatment group on Days 5 and 19, and the p-value of least square means difference from the 0 parts per million (ppm) dose.**

| Day | Dose (mg formalin/L) | Mean Percent Mortality* | p-value Least Square Means difference from 0 ppm |
|---|---|---|---|
| 5 | 0 | 65.9 | --- |
| 5 | 50 | 34.6 | 0.0018 |
| 5 | 100 | 25.7 | <0.0001 |
| 5 | 150 | 36.8 | 0.0037 |
| 19 | 0 | 68.1 | --- |
| 19 | 50 | 34.6 | 0.0014 |
| 19 | 100 | 27.9 | 0.0002 |
| 19 | 150 | 39.1 | 0.0054 |

*The mortality rate estimates listed are the least squares estimates from the statistical analysis.

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

**Conclusions:** The results of this study demonstrate the effectiveness of formalin applied at 50, 100, and 150 mg/L in 60-minute baths on alternate days for three treatments for the control of mortality in rainbow trout (*Oncorhynchus mykiss*) due to saprolegniasis associated with fungi in the family Saprolegniaceae.

2. Induced Infection Model Study

**Title:** GLP- Formalin treatment of fungal disease in catfish. Study No. 401-26.

**Study Dates:** April 7, 2006, to August 24, 2009

**Study Location:** Laurel, Maryland, U.S.A.

**Study Design:**

Objective: To evaluate formalin at concentrations of 0, 50, 100, and 150 mg/L to control mortality in channel catfish (*Ictalurus punctatus*) due to saprolegniasis due to fungi in the family Saprolegniaceae. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

Study Animals: Two hundred (200) channel catfish fingerlings were selected for the study. The study fish ranged from 13.2 to 17.1 centimeters in length and weighed 23.2 to 92.0 grams.

Experimental Design: Four trials were performed. For each trial, 50 fingerlings were used (48 experimental fish plus 2 extras). The fish were anesthetized with tricaine methanesulfonate and lightly abraded above the lateral line between the posterior margin of the dorsal fin and the anterior margin of the adipose fin using a mechanical scarification technique. The fish recovered from anesthesia before being placed into the exposure tank. Temperature stress was performed concurrently with fungal exposure by moving the catfish

from the acclimation temperature (24 ± 2 °C) to a lower exposure temperature (14 ± 2 °C). The fish were immersed together for 5-6 hours in a single bath exposure tank which had been inoculated with cultures of *Saprolegnia parasitica*. After exposure, fish were randomly distributed into individual compartments within 16 experimental (treatment) tanks (3 fish/tank). Each tank was randomly assigned to one of the treatment groups (0, 50, 100, and 150 mg/L formalin). Individual tank was the experimental unit.

Drug Administration: Treatments of 0, 50, 100, and 150 mg/L formalin were administered once daily every other day starting one hour after exposure ended for a total of three treatments. Each treatment lasted one hour. Fish were held for observation for two weeks post-treatment.

Measurements and Observations: All fish were monitored twice daily during the treatment and post-treatment observation period. A diagnosis of saprolegniasis was made visually based on the clinical manifestation of colony morphology on the fish. Moribund and dead fish were removed daily, examined grossly (the length and width of the lesion was measured, and the depth of the lesion was evaluated) and selected fish had tissues harvested for necropsy and histology. After the observation period, all surviving fish were euthanized, examined, and processed similarly to the dead and moribund fish. Water temperature, pH, and dissolved oxygen were recorded throughout the trials. Water samples, to verify formalin concentrations in the treatment tanks, were taken from the treatment water just before ending the 1-hour treatments.

**Statistical Methods:** Each trial was conducted using a randomized complete block design. In each of the four trials, 16 tanks were separated into 4 blocks, one treatment per block. Three fish were maintained and treated in each tank. Percent mortality per tank was calculated on Day 15. The arcsine-square root-transformed data was analyzed using a linear mixed model with fixed treatment effect and random effects trial, block within trial, and the interaction of treatment and trial.

**Results:** The treatment effect was statistically significant (P = 0.0077). Table 3 displays the comparison of each treatment least squares mean to the control least squares mean and the back-transformed means. Cumulative mortality results are included in Table 4. The majority of mortalities occurred by Day 8 in all trials.

The dissolved oxygen ranged from 7.9 to 11.8 mg/L. The pH ranged from 6.9 to 7.18. The water temperature of the exposure tanks ranged from 16 to 18 °C for Trial 1, 14-15 °C Trial 2, 13-14 °C Trial 3, and 12-14 °C during Trial 4. The temperature stress in Trial 1 was not as severe due to the malfunctioning of heat exchangers. The temperature of the treatment tanks was maintained at 24 ± 2 °C. The water quality results were acceptable for the culture of channel catfish.

Clinical manifestations of the fungus on the fish (hyphae and fluffy growths on the skin, fins, gills, or eyes) 2 to 4 days after exposure were used to verify the presence of infective fungus in the tank. All tanks contained fish with

fungal mats. Histology on dead and moribund fish confirmed the gross diagnosis. Of the 192 fish, only 8 did not have a grossly visible lesion. Histology on dead and moribund fish confirmed the gross diagnosis. Fungal hyphae were seen penetrating through the connective tissue of the dermis in some specimens.

**Table 3: Comparison of each treatment least squares mean to the control least squares mean and the back-transformed means.**

| Day | Dose (mg formalin/L) | P-value | Back-Transformed Least Squares Mean |
|---|---|---|---|
| 15 | 0 | n/a | 68.4% |
| 15 | 50 | 0.0566 | 50.0% |
| 15 | 100 | 0.0021 | 32.4% |
| 15 | 150 | 0.0036 | 35.5% |

**Table 4: Cumulative mortality on Day 15 following exposure.**

| Day | Dose (mg formalin /L) | Trial 1 | Trial 2 | Trial 3 | Trial 4 |
|---|---|---|---|---|---|
| 15 | 0 | 4 | 10 | 8 | 10 |
| 15 | 50 | 5 | 6 | 5 | 7 |
| 15 | 100 | 3 | 2 | 3 | 7 |
| 15 | 150 | 2 | 3 | 5 | 6 |

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

**Conclusions:** The results of this study demonstrate the effectiveness of formalin applied at 100 and 150 mg/L in 60-minute baths on alternate days for three treatments for the control of mortality in channel catfish (*Ictalurus punctatus*) due to saprolegniasis associated with fungi of the family Saprolegniaceae.

3. Supporting Information

The model studies described above support the effectiveness of both 100 and 150 mg/L in a 60-minute bath on alternate days for three treatments to control mortality on freshwater-reared finfish due to saprolegniasis. Based on a body of evidence from field data in the publicly disclosable Investigational New Animal Drug (INAD) files 9013 and 9346, and Public Master File (PMF) 5228, we concluded that there is support for a dose of 150 mg/L seen with treatment under clinical use conditions. These trials were not conducted under the standards of prospective clinical field trials but were supportive of the effectiveness of the higher dose of 150 mg/L and supported the safety and effectiveness of PARASITE-S in a variety of freshwater-reared fish species. No significant adverse drug events were reported during field use.

## III. TARGET ANIMAL SAFETY

CVM did not require target animal safety studies for this supplemental approval. The Freedom of Information (FOI) Summaries for the original approval of NADA 140-989 dated July 31, 1992, and the supplemental approval dated June 18, 1998, as well as the FOI Summaries of the PMF 3543 dated April 9, 1982, and PMF 5228 dated October 18, 1996, contain a summary of target animal safety studies for freshwater-reared finfish.

## IV. HUMAN FOOD SAFETY

### A. Antimicrobial Resistance

Formalin is not an antibacterial used in human clinical medicine against bacterial diseases; therefore, there were no requirements to address its impact on antimicrobial resistance development among bacteria of public health concern in or on formalin-treated fish.

### B. Effects of Residues on Human Intestinal Flora

Formalin is not an antibacterial used in human clinical medicine against bacterial diseases; therefore, there were no requirements to address its impact of formalin or its residues in or on edible tissues from treated fish on the intestinal flora of human consumers.

### C. Summary of Toxicology and Residue Chemistry Evaluation

CVM did not require new toxicology or residue chemistry studies for this supplemental approval. The FOI Summaries for the original approval of NADA 140-989 dated July 31, 1992, and for supplemental approvals dated September 30, 1993, and June 18, 1998, contain summaries of residue chemistry studies for a variety of aquatic species including salmon, trout, catfish, largemouth bass, striped bass, and shrimp. Because formalin treatment of this wide variety of aquatic species does not result in concentrations of formaldehyde in the edible tissues above the normal range of endogenous formaldehyde, formaldehyde is not expected to accumulate in additional finfish species that have not been specifically tested. It is expected that use of the drug in all finfish, all finfish eggs, and penaeid shrimp at the approved labeled concentrations will not result in the accumulation of formaldehyde above the normal range of endogenous formaldehyde in their edible tissue. Therefore, we have no human food safety concerns for the formaldehyde residues present in the edible tissue after treatment.

### D. Withdrawal Period:

A withdrawal period is not needed.

### E. Tolerance for Residues:

A tolerance is not needed.

### F. Analytical Method for Residues:

A regulatory method is not needed.

## V. USER SAFETY

Based on review of the literature (see key references listed below) on the potential of formalin (37% formaldehyde gas) to cause reproductive and teratogenic toxicities in humans, CVM has concluded that: (1) formalin has the potential to cause reproductive and teratogenic toxicities in humans if the exposure concentrations via inhalation exceed 0.5 ppm, the threshold for sensory irritation, and (2) formaldehyde is not likely to be inhaled by the user in concentrations above the sensory irritation level for over a sufficiently long period of time to cause reproductive and teratogenic effects. CVM expects that the use of Personal Protective Equipment (PPE) is adequate to prevent prolonged exposure at elevated levels and therefore mitigates the risk of reproductive and teratogenic toxicities.

References:


- Duong A, Steinmaus C, McHale CM, Vaughan CP, Zhang L. 2011. Reproductive and developmental toxicity of formaldehyde: a systematic review. Mutat Res Nov-Dec; 728(3):118-38.
- Thrasher JD, Kilburn KH. 2001. Embryo toxicity and teratogenicity of formaldehyde. Arch Environ Health Jul-Aug; 56(4):300-11.
- Zhou D, Zhang J, Wang H. 2011. Assessment of the potential reproductive toxicity of long-term exposure of adult male rats to low-dose formaldehyde. Toxicol Ind Health Aug; 27(7):591-598.
- National Toxicology Program (NTP). Formaldehyde, NTP Report on Carcinogens, Twelfth Edition, 2011.
- Environmental Protection Agency (EPA), Integrated Risk Information System (IRIS) Toxicological Review of Formaldehyde Inhalation Toxicity (Draft), 2010, EPA/635/R-10/002C.
- EPA, IRIS, 0419, Formaldehyde (CASRN 50-00-0). http://www.epa.gov/iris/subst/0419.htm
- Nielsen, GD, Larsen, ST, Wolkoff, P. 2013. Recent trend in risk assessment of formaldehyde exposures from indoor air. Arch Toxicology 87:73-78.
- McMartin KE, Martin-Amat G, Noker PE, Tephly TR. 1979. Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem Pharmacol 28(5): 645-649.


The product labeling contains the following information regarding safety to humans handling, administering, or exposed to PARASITE-S:

**USER SAFETY WARNINGS**
***Use Personal Protective Equipment (PPE) including eye, respiratory, and skin protection while handling this product. Refer to the SDS and OSHA regulations (29 CFR 1910.1048) for guidance on the most appropriate PPE equipment. Failure to use PPE may increase the risk of unsafe exposure to formaldehyde.***
Exposure to high concentrations of formaldehyde vapor causes severe respiratory irritation which can be life- threatening. Lower vapor levels can cause irritation to the eyes, respiratory tract, and skin. Swallowing formaldehyde can be life-threatening. Formaldehyde is an irritant when splashed on skin or into the eyes. It can cause severe eye damage, even blindness.
**Keep out of reach of children.**

In laboratory animals, formaldehyde has demonstrated the potential to cause reproductive and developmental toxicities at high dose. Use only with adequate ventilation. Keep container tightly closed when not in use.
May aggravate a pre-existing asthmatic condition and allergic rhinitis. Moderate fire and explosion hazard exists when exposed to heat or flame.
Contains methanol - cannot be made non-poisonous. Prolonged exposure to methanol has been associated with reproduction disorders.
May Cause Cancer: Formaldehyde vapor may be carcinogenic if inhaled. Use applicable safety protection. (Note: This drug, used as labeled, does not cause formaldehyde tissue residues in fish).
Employers: Refer to Occupational Safety and Health Administration (OSHA) regulation 29 CFR 1910.1048 for human safety guidance that may be applicable to your specific operation. OSHA's "action level" concentration or airborne formaldehyde is 0.5 part per million (ppm), calculated as an 8-hour time-weighted average (TWA). Use respiratory, skin, and eye protection when needed (refer to OSHA's regulation 29 CFR 1910.1048). OSHA's airborne exposure limits (without use of a respirator) for formaldehyde shall not exceed 1) 0.75 part per million (ppm) as an 8-hour, time- weighted average (TWA) or 2) 2 parts per million (ppm) as a 15-minute, short term exposure limit (STEL). **NOTE:** The odor of formaldehyde in the air can generally be detected at about 0.5 to 0.8 ppm (range about 0.05 to 1 ppm).

**USER EXPOSURE EMERGENCY AID**
**INHALATION (Breathing)**: Get medical aid immediately. Remove victim from exposure wearing protective clothing and respiratory protection appropriate to the type and degree of contamination. Move victim to fresh air immediately. If breathing is difficult, give oxygen. DO NOT use mouth-to-mouth respiration. If breathing has ceased, induce artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. If no respiratory device is available, perform chest compressions only.
**INGESTION (Swallowing)**: DO NOT induce vomiting. If the person is conscious, dilute, inactivate, or absorb the formaldehyde by giving milk, activated charcoal, or water. Get medical help immediately. If vomiting occurs, keep head lower than hips.
**EYE CONTACT**: Immediately flush eye(s) with large amounts of water for at least 15 minutes, lifting the lower and upper eyelids occasionally, until no evidence of chemical remains. Seek medical attention immediately. DO NOT allow victim to rub eyes or keep eyes closed for burns to eyes may have a delayed effect.
**SKIN CONTACT**: Remove contaminated clothing (including shoes) immediately. Wash affected area of body with soap and large amounts of water until no evidence of chemical remains (at least 15 minutes). If there are chemical burns, or appreciable eye or respiratory irritation, get medical help immediately.

## 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 PARASITE-S, when used according to the label, is safe and effective for the control of mortality in freshwater-reared finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae. Additionally, data demonstrate that residues in food products derived from species treated with PARASITE-S 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 PARASITE-S 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 PARASITE-S. PARASITE-S, as approved in our letter, does not qualify for marketing exclusivity under section 512(c)(2)(F) of the FD&C Act.

### C. Supplemental Applications

This supplemental NADA required 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.